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(54) Title: <b>62 HUMAN SECRETED PROTEINS</b>			
(57) Abstract <p>The present invention relates to novel human secreted proteins and isolated nucleic acids containing the coding regions of the genes encoding such proteins. Also provided are vectors, host cells, antibodies, and recombinant methods for producing human secreted proteins. The invention further relates to diagnostic and therapeutic methods useful for diagnosing and treating diseases, disorders, and/or conditions related to these novel human secreted proteins.</p>			

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## 62 Human Secreted Proteins

### *Field of the Invention*

This invention relates to newly identified polynucleotides and the polypeptides encoded by these polynucleotides, uses of such polynucleotides and polypeptides, and their production.

### *Background of the Invention*

Unlike bacterium, which exist as a single compartment surrounded by a membrane, human cells and other eucaryotes are subdivided by membranes into many functionally distinct compartments. Each membrane-bounded compartment, or organelle, contains different proteins essential for the function of the organelle. The cell uses "sorting signals," which are amino acid motifs located within the protein, to target proteins to particular cellular organelles.

One type of sorting signal, called a signal sequence, a signal peptide, or a leader sequence, directs a class of proteins to an organelle called the endoplasmic reticulum (ER). The ER separates the membrane-bounded proteins from all other types of proteins. Once localized to the ER, both groups of proteins can be further directed to another organelle called the Golgi apparatus. Here, the Golgi distributes the proteins to vesicles, including secretory vesicles, the cell membrane, lysosomes, and the other organelles.

Proteins targeted to the ER by a signal sequence can be released into the extracellular space as a secreted protein. For example, vesicles containing secreted proteins can fuse with the cell membrane and release their contents into the extracellular space - a process called exocytosis. Exocytosis can occur constitutively or after receipt of a triggering signal. In the latter case, the proteins are stored in secretory vesicles (or secretory granules) until exocytosis is triggered. Similarly,

proteins residing on the cell membrane can also be secreted into the extracellular space by proteolytic cleavage of a "linker" holding the protein to the membrane.

Despite the great progress made in recent years, only a small number of genes encoding human secreted proteins have been identified. These secreted proteins  
5 include the commercially valuable human insulin, interferon, Factor VIII, human growth hormone, tissue plasminogen activator, and erythropoietin. Thus, in light of the pervasive role of secreted proteins in human physiology, a need exists for identifying and characterizing novel human secreted proteins and the genes that encode them. This knowledge will allow one to detect, to treat, and to prevent  
10 medical diseases, disorders, and/or conditions by using secreted proteins or the genes that encode them.

### ***Summary of the Invention***

The present invention relates to novel polynucleotides and the encoded  
15 polypeptides. Moreover, the present invention relates to vectors, host cells, antibodies, and recombinant and synthetic methods for producing the polypeptides and polynucleotides. Also provided are diagnostic methods for detecting diseases, disorders, and/or conditions related to the polypeptides and polynucleotides, and therapeutic methods for treating such diseases, disorders, and/or conditions. The  
20 invention further relates to screening methods for identifying binding partners of the polypeptides.

### ***Detailed Description***

#### **Definitions**

25 The following definitions are provided to facilitate understanding of certain terms used throughout this specification.

In the present invention, "isolated" refers to material removed from its original environment (e.g., the natural environment if it is naturally occurring), and thus is altered "by the hand of man" from its natural state. For example, an isolated polynucleotide could be part of a vector or a composition of matter, or could be  
5 contained within a cell, and still be "isolated" because that vector, composition of matter, or particular cell is not the original environment of the polynucleotide. The term "isolated" does not refer to genomic or cDNA libraries, whole cell total or mRNA preparations, genomic DNA preparations (including those separated by electrophoresis and transferred onto blots), sheared whole cell genomic DNA  
10 preparations or other compositions where the art demonstrates no distinguishing features of the polynucleotide/sequences of the present invention.

In the present invention, a "secreted" protein refers to those proteins capable of being directed to the ER, secretory vesicles, or the extracellular space as a result of  
15 a signal sequence, as well as those proteins released into the extracellular space without necessarily containing a signal sequence. If the secreted protein is released into the extracellular space, the secreted protein can undergo extracellular processing to produce a "mature" protein. Release into the extracellular space can occur by many mechanisms, including exocytosis and proteolytic cleavage.

20 In specific embodiments, the polynucleotides of the invention are at least 15, at least 30, at least 50, at least 100, at least 125, at least 500, or at least 1000 continuous nucleotides but are less than or equal to 300 kb, 200 kb, 100 kb, 50 kb, 15 kb, 10 kb, 7.5 kb, 5 kb, 2.5 kb, 2.0 kb, or 1 kb, in length. In a further embodiment, polynucleotides of the invention comprise a portion of the coding sequences, as  
25 disclosed herein, but do not comprise all or a portion of any intron. In another embodiment, the polynucleotides comprising coding sequences do not contain coding

sequences of a genomic flanking gene (i.e., 5' or 3' to the gene of interest in the genome). In other embodiments, the polynucleotides of the invention do not contain the coding sequence of more than 1000, 500, 250, 100, 50, 25, 20, 15, 10, 5, 4, 3, 2, or 1 genomic flanking gene(s).

5           As used herein, a "polynucleotide" refers to a molecule having a nucleic acid sequence contained in SEQ ID NO:X or the cDNA contained within the clone deposited with the ATCC. For example, the polynucleotide can contain the nucleotide sequence of the full length cDNA sequence, including the 5' and 3' untranslated sequences, the coding region, with or without the signal sequence, the  
10   secreted protein coding region, as well as fragments, epitopes, domains, and variants of the nucleic acid sequence. Moreover, as used herein, a "polypeptide" refers to a molecule having the translated amino acid sequence generated from the polynucleotide as broadly defined.

          In the present invention, the full length sequence identified as SEQ ID NO:X  
15   was often generated by overlapping sequences contained in multiple clones (contig analysis). A representative clone containing all or most of the sequence for SEQ ID NO:X was deposited with the American Type Culture Collection ("ATCC"). As shown in Table 1, each clone is identified by a cDNA Clone ID (Identifier) and the ATCC Deposit Number. The ATCC is located at 10801 University Boulevard,  
20   Manassas, Virginia 20110-2209, USA. The ATCC deposit was made pursuant to the terms of the Budapest Treaty on the international recognition of the deposit of microorganisms for purposes of patent procedure.

          A "polynucleotide" of the present invention also includes those polynucleotides capable of hybridizing, under stringent hybridization conditions, to  
25   sequences contained in SEQ ID NO:X, the complement thereof, or the cDNA within the clone deposited with the ATCC. "Stringent hybridization conditions" refers to an

overnight incubation at 42 degree C in a solution comprising 50% formamide, 5x SSC (750 mM NaCl, 75 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20  $\mu$ g/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65 degree C.

- 5           Also contemplated are nucleic acid molecules that hybridize to the polynucleotides of the present invention at lower stringency hybridization conditions. Changes in the stringency of hybridization and signal detection are primarily accomplished through the manipulation of formamide concentration (lower percentages of formamide result in lowered stringency); salt conditions, or
- 10   temperature. For example, lower stringency conditions include an overnight incubation at 37 degree C in a solution comprising 6X SSPE (20X SSPE = 3M NaCl; 0.2M  $\text{NaH}_2\text{PO}_4$ ; 0.02M EDTA, pH 7.4), 0.5% SDS, 30% formamide, 100  $\mu$ g/ml salmon sperm blocking DNA; followed by washes at 50 degree C with 1XSSPE, 0.1% SDS. In addition, to achieve even lower stringency, washes performed
- 15   following stringent hybridization can be done at higher salt concentrations (e.g. 5X SSC).

- Note that variations in the above conditions may be accomplished through the inclusion and/or substitution of alternate blocking reagents used to suppress background in hybridization experiments. Typical blocking reagents include
- 20   Denhardt's reagent, BLOTTO, heparin, denatured salmon sperm DNA, and commercially available proprietary formulations. The inclusion of specific blocking reagents may require modification of the hybridization conditions described above, due to problems with compatibility.

- Of course, a polynucleotide which hybridizes only to polyA+ sequences (such
- 25   as any 3' terminal polyA+ tract of a cDNA shown in the sequence listing), or to a

complementary stretch of T (or U) residues, would not be included in the definition of "polynucleotide," since such a polynucleotide would hybridize to any nucleic acid molecule containing a poly (A) stretch or the complement thereof (e.g., practically any double-stranded cDNA clone generated using oligo dT as a primer).

5           The polynucleotide of the present invention can be composed of any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. For example, polynucleotides can be composed of single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of  
10 single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, the polynucleotide can be composed of triple-stranded regions comprising RNA or DNA or both RNA and DNA. A polynucleotide may also contain one or more modified bases or DNA or RNA  
15 backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications can be made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically, or metabolically modified forms.

          The polypeptide of the present invention can be composed of amino acids  
20 joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres, and may contain amino acids other than the 20 gene-encoded amino acids. The polypeptides may be modified by either natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more  
25 detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid

side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched, for example, as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched, and branched cyclic polypeptides may result from posttranslation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, pegylation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. (See, for instance, PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York (1993); POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, pgs. 1-12 (1983); Seifter et al., Meth Enzymol 182:626-646 (1990); Rattan et al., Ann NY Acad Sci 663:48-62 (1992).)

"SEQ ID NO:X" refers to a polynucleotide sequence while "SEQ ID NO:Y" refers to a polypeptide sequence, both sequences identified by an integer specified in Table 1.

"A polypeptide having biological activity" refers to polypeptides exhibiting activity similar, but not necessarily identical to, an activity of a polypeptide of the

present invention, including mature forms, as measured in a particular biological assay, with or without dose dependency. In the case where dose dependency does exist, it need not be identical to that of the polypeptide, but rather substantially similar to the dose-dependence in a given activity as compared to the polypeptide of the present invention (i.e., the candidate polypeptide will exhibit greater activity or not more than about 25-fold less and, preferably, not more than about tenfold less activity, and most preferably, not more than about three-fold less activity relative to the polypeptide of the present invention.)

Many proteins (and translated DNA sequences) contain regions where the amino acid composition is highly biased toward a small subset of the available residues. For example, membrane spanning domains and signal peptides (which are also membrane spanning) typically contain long stretches where Leucine (L), Valine (V), Alanine (A), and Isoleucine (I) predominate. Poly-Adenosine tracts (polyA) at the end of cDNAs appear in forward translations as poly-Lysine (poly-K) and poly-Phenylalanine (poly-F) when the reverse complement is translated. These regions are often referred to as "low complexity" regions.

Such regions can cause database similarity search programs such as BLAST to find high-scoring sequence matches that do not imply true homology. The problem is exacerbated by the fact that most weight matrices (used to score the alignments generated by BLAST) give a match between any of a group of hydrophobic amino acids (L,V and I) that are commonly found in certain low complexity regions almost as high a score as for exact matches.

In order to compensate for this, BLASTX.2 (version 2.0a5MP-WashU) employs two filters ("seg" and "xnu") which "mask" the low complexity regions in a particular sequence. These filters parse the sequence for such regions, and create a new sequence in which the amino acids in the low complexity region have been



replaced with the character "X". This is then used as the input sequence (sometimes referred to herein as "Query" and/or "Q") to the BLASTX program. While this regime helps to ensure that high-scoring matches represent true homology, there is a negative consequence in that the BLASTX program uses the query sequence that has  
5 been masked by the filters to draw alignments.

Thus, a stretch of "X"s in an alignment shown in the following application does not necessarily indicate that either the underlying DNA sequence or the translated protein sequence is unknown or uncertain. Nor is the presence of such stretches meant to indicate that the sequence is identical or not identical to the  
10 sequence disclosed in the alignment of the present invention. Such stretches may simply indicate that the BLASTX program masked amino acids in that region due to the detection of a low complexity region, as defined above. In all cases, the reference sequence(s) (sometimes referred to herein as "Subject", "Sbjct", and/or "S") indicated in the specification, sequence table (Table I), and/or the deposited clone is (are) the  
15 definitive embodiment(s) of the present invention, and should not be construed as limiting the present invention to the partial sequence shown in an alignment, unless specifically noted otherwise herein.

## 20 **Polynucleotides and Polypeptides of the Invention**

### **FEATURES OF PROTEIN ENCODED BY GENE NO: 1**

The translation product of this gene shares sequence homology with a  
25 metalloproteinase (accession AJ003144) from Homo sapiens. ProSite similarities

suggest a zinc metalloprotease. The alignment demonstrating this similarity is shown below:

```

5   >gnl|PID|e1245446 (AJ003144) metalloproteinase [Homo sapiens]
    >gnl|PID|e1246030
      (AJ003147) metalloproteinase [Homo sapiens] >gnl|PID|e1246030
      (AJ003147) metalloproteinase [Homo sapiens] >sp|O43923|O43923
      METALLOPROTEINASE.
      Length = 183
10
    Plus Strand HSPs:

    Score = 683 (240.4 bits), Expect = 1.3e-66, P = 1.3e-66
    Identities = 128/144 (88%), Positives = 128/144 (88%), Frame = +2
15
    Query:   560 MDPGTVATMRKPRCSLPDXXXXXXXXXXXXXXXXXSGSVWKKRTL TWRVRSFPQSSQLSQE
739
           MDPGTVATMRKPRCSLPD                      SGSVWKKRTL TWRVRSFPQSSQLSQE
    Sbjct:   1  MDPGTVATMRKPRCSLPDVLGVAGLVRRRRRYALSGSVWKKRTL TWRVRSFPQSSQLSQE
20  60

    Query:   740 TVRVLM SYALMAWGMESGLTFHEVDSPQGQEPDILIDFARAFHQDSYPFDGLGGTLAHAF
919
           TVRVLM SYALMAWGMESGLTFHEVDSPQGQEPDILIDFARAFHQDSYPFDGLGGTLAHAF
25  Sbjct:   61 TVRVLM SYALMAWGMESGLTFHEVDSPQGQEPDILIDFARAFHQDSYPFDGLGGTLAHAF
120

    Query:   920 FPGEHPISGDTHFDDEETWTFGSK 991
           FPGEHPISGDTHFDDEETWTFGSK
30  Sbjct:   121 FPGEHPISGDTHFDDEETWTFGSK 144

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The homologous gene (which is shorter than our protein) was found in the FMF (Familial Mediterranean Fever a hereditary disorder that affects certain Jews, Armenians, Turks and Arabs) region of chromosome 16 (Bernot et. al (1998)

35 Genomics 50(2):147-60.

Preferred polypeptides of the invention comprise the following amino acid sequence:

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HASGWRTPRDPERPPRHIQTSAAPAPSQPSWDSRAHPTQRRDPGPPGPSADST
AHFPGPPHTSQPSGRSLPTRCRVPPALSRPGSPPPGPRGGPSQAPFERRRPGL
40 GRT (SEQ ID NO: 163),
HASGWRTPRDPERPPRHIQTSAAPAPSQPSWDSRAHPTQRRDPGPPGPSADST

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AHF (SEQ ID NO: 164), and/or

PGPPHTSQPSGRSLPTRCRVPPALSRPGSPPPGPRGGPSQAPFEPRRRPGLGRT

(SEQ ID NO: 165). Polynucleotides encoding these polypeptides are also encompassed by the invention.

- 5           The translation product of this gene contains a zinc-binding region signature Pattern: [GSTALIVN].{2}HE[LIVMFYW][<sup>^</sup>DEHRKP]H.[LIVMFYWGSPQ] VAVHEFGHAL. Amino acids that comprise this zinc-binding signature are also preferred nonexclusive embodiments of the invention.

10           This gene is expressed primarily in Human eosinophils and primary dendritic cells.

          Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, FMF (Familial Mediterranean Fever), as well as disorders of the immune system. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, especially dendritic cells and eosinophils, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder. Preferred polypeptides of the present invention comprise immunogenic epitopes shown in SEQ ID NO: 87 as residues: Ala-19 to Ala-26, Gln-53 to Leu-58, Glu-73 to Pro-79, Val-114 to Thr-119,

15

20

25

Ser-126 to Ser-134, Val-160 to Pro-168, Phe-178 to Asp-186, Ser-204 to Trp-215, Gly-218 to Gly-224. Polynucleotides encoding said polypeptides are also provided.

The tissue distribution in immune tissues, and the homology to a metalloproteinase, indicates that polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and/or treatment of Familial Mediterranean Fever (FMF). Furthermore, expression of this gene product in tissues and cells of the immune system indicates a role in the regulation of the proliferation; survival; differentiation; and/or activation of potentially all hematopoietic cell lineages, including blood stem cells. This gene product may be involved in the regulation of cytokine production, antigen presentation, or other processes that may also suggest a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the gene or protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immune deficiency diseases such as AIDS, leukemia, rheumatoid arthritis, inflammatory bowel disease, sepsis, acne, and psoriasis. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:11 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is

cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1524 of SEQ ID NO:11, b is an integer of 15 to 1538, where both a and b correspond to the positions of nucleotide  
5 residues shown in SEQ ID NO:11, and where b is greater than or equal to a + 14.

## **FEATURES OF PROTEIN ENCODED BY GENE NO: 2**

10           Based on homology this gene encodes a human umbilical cord vein endothelial cell polypeptide (See Geneseq Accession No. T03018).

          When tested against U937 Myeloid cell lines, supernatants removed from cells containing this gene activated the GAS assay. Thus, it is likely that this gene activates myeloid cells through the Jak-STAT signal transduction pathway. The gamma  
15 activating sequence (GAS) is a promoter element found upstream of many genes which are involved in the Jak-STAT pathway. The Jak-STAT pathway is a large, signal transduction pathway involved in the differentiation and proliferation of cells. Therefore, activation of the Jak-STAT pathway, reflected by the binding of the GAS element, can be used to indicate proteins involved in the proliferation and  
20 differentiation of cells.

          This gene is expressed primarily in endothelial cells, including vascular rich tissues such as umbilical vein endothelial cells and aortic endothelium.

          Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a  
25 biological sample and for diagnosis of diseases and conditions which include, but are not limited to, pathologies of the cardiovascular system. Similarly, polypeptides and

antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the cardiovascular system, expression of this gene at significantly higher or lower levels may be routinely  
5 detected in certain tissues or cell types (e.g., vascular, endothelial, hematopoietic, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the  
10 disorder. Preferred polypeptides of the present invention comprise immunogenic epitopes shown in SEQ ID NO: 88 as residues: Gly-21 to Gly-36, Pro-47 to Gly-53, Ser-58 to His-65, Ser-78 to Thr-89, Val-93 to Thr-98, Phe-149 to Ser-165, Ala-175 to Asp-180, Asn-193 to Ser-200. Polynucleotides encoding said polypeptides are also provided.

15       Based upon sequence homology, this gene is known to be a human umbilical cord vein endothelial cell polypeptide, with tissue distribution in vascular rich endothelial tissues.

      The protein product of this gene showed biological activity in the GAS assay when tested against U937 myeloid cell lines. Thus, it is likely that this gene activates  
20 myeloid cells, and other hematopoietic cells, through the Jak-STAT signal transduction pathway. The gamma activating sequence (GAS) is a promoter element found upstream of many genes which are involved in the Jak-STAT pathway. The Jak-STAT pathway is a large, signal transduction pathway involved in the differentiation and proliferation of cells.

25       Polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and/or treatment of disorders involving the vasculature. Elevated expression

of this gene product by endothelial cells, and the biological activity of the protein product of this gene indicates that it may play vital roles in the regulation of endothelial cell function, secretion, or proliferation.

Alternately, this may represent a gene product expressed by the endothelium  
5 and transported to distant sites of action on a variety of target organs. Expression of this gene product at elevated levels in both endothelial cells and biological activity of this gene product on hematopoietic cells is consistent with the common ancestry of these two lineages, and indicates roles for the gene product in a variety of processes, including vasculogenesis, angiogenesis, survival, differentiation, and proliferation of  
10 blood cell lineages, and normal immune function and immune surveillance. Biological activity of this gene product on hematopoietic cells also indicates involvement in the proliferation, survival, activation, or differentiation of all blood cell lineages. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

15 Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:12 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is  
20 cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1033 of SEQ ID NO:12, b is an integer of 15 to 1047, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:12, and where b is greater than or equal to a + 14.

**FEATURES OF PROTEIN ENCODED BY GENE NO: 3**

The translation product of this gene shares sequence homology with *Drosophila* slit-2 and other EGF-repeat containing extracellular and cell surface proteins which are known to be important in neuron guidance, embryonic development and tissue repair. The translation product of this gene is believed to have similar biological activities based on the sequence similarity to these known proteins. Such activities are known in the art and can be routinely assayed by well known techniques.

Preferred polypeptides of the invention comprise: the extracellular domain (residues 24-576), the transmembrane domain (residues 577-593), and/or the intracellular domain (residues 594-672) as shown in the polypeptide sequence in the sequence listing identified for this gene in Table 1. Other preferred polypeptides comprise one or more of the multiple EGF repeats present in this protein.

Polynucleotides encoding such polypeptides are also encompassed by the invention.

Preferred polypeptides comprise the following amino acid sequence:

HASASGRVDADSNASGPRTPSGPTRQERLRPRPAPPSLRRRRLPGQKM  
 CSRVPLLLPLLLLLALGPGVQGCPSGCQCSQPQTVFCTARQGTTVPRDVPPDT  
 VGLYVFENGITMLDAGSFAGLPGLQLLDLSQNQIASLPSGVFQPLANLSNLDL  
 TANRLHEITNETFRGLRRLERLYLGKNRIRHIQPGAFDTLDRLLELKLQDNEL  
 RALPPLRLPRLLLLDLSHNSLLALEPGILDTANVEALRLAGLGLQQLDEGLFSR  
 LRNLHDLVDSDNQLERVPPVIRGLRGLTRLRLAGNTRIAQLRPEDLAGLAAL  
 QELDVSNSLSLQALPGDLSGLFPRLRLAAARNPFNCVCPLSWFGPWVRESHV  
 TLASPEETRCHFPPKNAGRLLLELDYADFGCPATTTTATVPTTRPVVREPT  
 ALSSSLAPTWLSPTAPATEAPSPSTAPPTVGPVPQPQDCPPSTCLNGGTCHLG  
 TRHHLACLCPEGFTGLYCESQMGQGTSPVTPRPPRSLTLGIEPVSPSLR



VGLQRYLQGSSVQLRSLRLTYRNLSGPDKRLVTLRLPASLAEYTVTQLRPNA  
 TYSVCVMPLGPGRVPEGEEACGEAHTPPAVHSNHAPVTQAREGNLPLLIAPA  
 LAAVLLAALAAVGAAYCVRRGRAMAAAAQDKGQVGPGAGPLELEGVKVPL  
 EPGPKATEAVERPCPAGLSVKCHSWASKAWPQSPLHAKPYI (SEQ ID NO:

5 166) Also preferred are the polynucleotides encoding these polypeptides.

The gene encoding the disclosed cDNA is believed to reside on chromosome  
 5. Accordingly, polynucleotides related to this invention are useful as a marker in  
 linkage analysis for chromosome 5.

This gene is expressed primarily in osteoblasts and CD34-depleted cord blood  
 10 and to a lesser extent in some other, predominantly hematopoietic organs.

Therefore, polynucleotides and polypeptides of the invention are useful as  
 reagents for differential identification of the tissue(s) or cell type(s) present in a  
 biological sample and for diagnosis of diseases and conditions which include, but are  
 not limited to, growth and immune defects. Similarly, polypeptides and antibodies  
 15 directed to these polypeptides are useful in providing immunological probes for  
 differential identification of the tissue(s) or cell type(s). For a number of disorders of  
 the above tissues or cells, particularly of the musculoskeletal and hematopoietic  
 systems, expression of this gene at significantly higher or lower levels may be  
 routinely detected in certain tissues or cell types (e.g., cancerous and wounded  
 20 tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or  
 another tissue or cell sample taken from an individual having such a disorder, relative  
 to the standard gene expression level, i.e., the expression level in healthy tissue or  
 bodily fluid from an individual not having the disorder. Preferred polypeptides of the  
 present invention comprise immunogenic epitopes shown in SEQ ID NO: 89 as  
 25 residues: Cys-28 to Pro-33, Arg-41 to Pro-52, Glu-118 to Glu-127, Tyr-130 to Arg-  
 135, Ser-224 to Arg-230, Ser-322 to His-329, Glu-388 to Ala-396, Pro-404 to Pro-

411, Ser-443 to Thr-454, Val-456 to Arg-462, Asn-500 to Arg-507. Polynucleotides encoding said polypeptides are also provided.

The tissue distribution and homology to slit protein indicates that polynucleotides and polypeptides corresponding to this gene are useful for diagnosis, study and treatment of musculoskeletal and other developmental disorders, immune and blood conditions and wound healing.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:13 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2787 of SEQ ID NO:13, b is an integer of 15 to 2801, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:13, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 4

20

Preferred polypeptides of the invention comprise the following amino acid sequence:

HASGRLQTQREGGQGVGRRRTEEGTETQSKGGKEETLVGGRHSGERGGWAE  
(SEQ ID NO: 167). Polynucleotides encoding these polypeptides are also

25 encompassed by the invention.

This gene is expressed primarily in heart, parathyroid tumor, larynx tumor, ovarian tumor, keratinocytes, healing ground wound tissue, and epithelial tissues, and to a lesser extent in many other tissues.

Therefore, polynucleotides and polypeptides of the invention are useful as  
5 reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, cardiovascular diseases, endocrine disorders, reproductive disorders, epithelial disorders or tumors. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential  
10 identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the cardiovascular, epithelial, endocrine, reproductive systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., reproductive, endocrine, epithelial, cancerous and wounded tissues) or bodily fluids (e.g., lymph, amniotic  
15 fluid, bile, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder. Preferred polypeptides of the present invention comprise immunogenic epitopes shown in SEQ ID NO: 90 as residues: Gln-25 to  
20 Gly-32, Gly-159 to Gly-167, Gln-195 to Thr-208, Ala-222 to Cys-330, Lys-332 to Gly-341, Gln-346 to Ser-351, Asn-377 to Pro-386. Polynucleotides encoding said polypeptides are also provided.

The tissue distribution in heart, parathyroid tumor, larynx tumor, and ovarian tumor indicates that polynucleotides and polypeptides corresponding to this gene are  
25 useful for treatment or diagnosis of cardiovascular diseases, epithelial, endocrine disorders, and/or reproductive disorders.

The tissue distribution in smooth muscle tissue indicates that the protein product of this gene is useful for the diagnosis and treatment of conditions and pathologies of the cardiovascular system, such as heart failure, congenital heart diseases, ischemic heart diseases, restenosis, atherosclerosis, stroke, angina,

5 thrombosis, rheumatic/hypersensitivity diseases, cardiomyopathy, luetic heart disease, inflammatory diseases of the heart, hypertensive heart disease, nutritional, endocrine, and metabolic diseases of the heart and wound healing.

The tissue distribution in epithelial tissue and healing groin wound tissues indicates that the translation product of this gene is useful for the diagnosis, detection

10 and/or treatment of diseases and/or disorders involving epithelial tissues, such as infections and wound healing disorders, for example, as is disclosed in more detail herein. Additionally, polynucleotides and polypeptides corresponding to this gene are useful for the detection, treatment, and/or prevention of various endocrine disorders and cancers, particularly Addison's disease, Cushing's Syndrome, and disorders

15 and/or cancers of the pancreas (e.g., diabetes mellitus), adrenal cortex, ovaries, pituitary (e.g., hyper-, hypopituitarism), thyroid (e.g., hyper-, hypothyroidism), parathyroid (e.g., hyper-, hypoparathyroidism), hypothalamus, and testes. Similarly, polynucleotides and polypeptides corresponding to this gene are useful for the treatment and diagnosis of conditions concerning proper ovary function (e.g.,

20 endocrine function, egg maturation), as well as cancer (e.g., ovarian tumors, serous adenocarcinoma, dysgerminoma, embryonal carcinoma, choriocarcinoma, teratoma, etc.). Therefore, this gene product is useful in the treatment of female infertility, sexual dysfunction or sex development disorders. Similarly, the protein is believed to be useful in the treatment and/or diagnosis of ovarian cancer. Protein, as well as,

25 antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:14 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically

5 excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1427 of SEQ ID NO:14, b is an integer of 15 to 1441, where both a and b correspond to the positions of nucleotide

10 residues shown in SEQ ID NO:14, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 5**

15 The polynucleotide sequence of this gene may have a frame shift. Therefore, preferred polypeptides of the invention comprise the following amino acid sequence:

PRVRAESEGTYDTYQHVPVESFAEVLLRTGKLAEAKNKGEVFPTEVLLQLA  
SEALPND (SEQ ID NO: 168),

LERLLRQSNILLRSPRKRNSEDEAQEAKDSKVTYADTLNHLEKSLAHLETLSH

20 SFILSLKNSEQETLQKYSHLYDLRSEKEKHLHDEAVAICLDGQPLAMIQQLLE  
VAVGPLDISPKDIVQSAIMKIISALSGGSADLGGPRDPLKVLEGVVAHVHASV  
DKGEELVSPEDLLEWLRPFCADDAWPVRPRIHVLQILGQSFHLTEEDSKLLVF  
FRTEAILKASWPQRQVDIADIENEENRYCLFMELLESSHHEAEFQHLVLLLQA  
WPPMKSEYVITNNPWVRLATVMLTRCTMENKEGLGNEVLKMCRSLYNTKQ

25 MLPAEGVKELCLLLLNQSLLLPSLKLLLESRDEHLHEMALEQITAVTTVNDNSN  
CDQELLSLLLDKLLVKCVSTPFYPRIVDHLLASLQQGRWDAEELGRHLREA

- GHEAEAGSLLLA VRGTHQAFRTFSTALRAAQHWV (SEQ ID NO: 169),  
LERLLRQSNILLRSPRKRNSEDEAQEAKDSKVTYAD (SEQ ID NO: 170),  
TLNHLEKSLAHLETLSHSFILSLKNSEQETLQKYS (SEQ ID NO: 171),  
HLYDLRSEKEKLHDEAVAICLDGQPLAMIQQLLEV (SEQ ID NO: 172),  
5 AVGPLDISPKDIVQSAIMKIISALSGGSADLGGPR (SEQ ID NO: 173),  
DPLKVLEGVVA AVHASVDKGEEVSPEDLLEWLRPF (SEQ ID NO: 174),  
CADDAPVVRPRIHV LQILGQSFHLTEEDSKLLVFF (SEQ ID NO: 175),  
RTEAILKASWPQRQVDIADIENEENRYCLFMELLESS (SEQ ID NO: 176),  
HHEAEFQHLVLLLQAWPPMKSEYVITNNPWVRLA (SEQ ID NO: 177),  
10 TVMLTRCTMENKEGLGNEVLKMCRSLYNTKQMLPAE (SEQ ID NO: 178),  
GVKELCLLLL NQSLLLPSLKLLESRDEHLHEMAL (SEQ ID NO: 179),  
EQITAVTTVNDSNCDQELLSLLD A KLLVKCVSTPF (SEQ ID NO: 180),  
YPRIVDHLLASLQQGRWD AEELGRHLREAGHEAEA (SEQ ID NO: 181),  
GSLLLAVRGTHQAFRTFSTALRAAQHWV (SEQ ID NO: 182),  
15 PSSYTATMNVSWISLRRRSFRAFG RVWTC SGLLQMTSIKGKLSLVWQRLDGH  
FCRTLEESVYSIAISLAQRYSVSRWEVFMTHLEFLFTDSGLSTLEIENRAQDLH  
LFETLKTDP EAFHQH MVKYIYPTIGGFDHERLQYYFTLLENCGCADLG NCAIK  
PETHIRLLKKFKVVASGLNYKKLT DENMSPLEALEPVLSSQNILSISKLVPKIPE  
KDGQMLSPSSLYTIWLQKLFWTGDPHLIKQVPGSSPEWLHAYDVC MKYFDR  
20 LHPGDLITVVD AVTFSPKAVTKLSVEARKEMTRKAIKTVKHFIEKPKEKKLRR  
RSSRS (SEQ ID NO: 183),  
PSSYTATMNVSWISLRRRSFRAFG RVWTC SGLLQMTSI (SEQ ID NO: 184),  
KGKLSLVWQRLDGHFCRTLEESVYSIAISLAQR (SEQ ID NO: 185),  
YSVSRWEVFMTHLEFLFTDSGLSTLEIENRAQDLH (SEQ ID NO: 186),  
25 LFETLKTDP EAFHQH MVKYIYPTIGGFDHERLQYYF (SEQ ID NO: 187),  
TLENCGCADLG NCAIKPETHIRLLKKFKVVASGL (SEQ ID NO: 188),

NYKKLTDENMSPLEALEPVLSSQNILSISKLVPKIP (SEQ ID NO: 189),  
EKDGQMLSPSSLYTIWLQKLFWTGDPHLIKQVPGSS (SEQ ID NO: 190),  
PEWLHAYDVCMKYFDR LH PGDLITVVD AVTFSPKA (SEQ ID NO: 191), and/or  
VTKLSVEARKEMTRKAIKTVKHFIEKPKEKKLRRRSSRS (SEQ ID NO: 192).

- 5 Polynucleotides encoding these polypeptides are also encompassed by the invention.

The gene encoding the disclosed cDNA is believed to reside on chromosome 2. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 2.

- This gene is expressed primarily in fetal liver spleen, human tonsils, placenta,  
10 pancreas islet cell tumor, chronic lymphocytic leukemia, primary dendritic cells.

- Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, pancreas islet cell tumor, chronic lymphocytic leukemia. Similarly,  
15 polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune and hematopoietic, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., hematopoietic, immune,  
20 cancerous and wounded tissues) or bodily fluids (e.g., lymph, amniotic fluid, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder. Preferred polypeptides of the present invention comprise  
25 immunogenic epitopes shown in SEQ ID NO: 91 as residues: Phe-21 to Pro-26. Polynucleotides encoding said polypeptides are also provided.

The tissue distribution in fetal liver and spleen tissues indicates that polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and/or treatment of hematopoietic disorders. This gene product is primarily expressed in hematopoietic cells and tissues, suggesting that it plays a role in the survival, proliferation, and/or differentiation of hematopoietic lineages. This is particularly supported by the expression of this gene product in fetal liver, one of the two primary sites of definitive hematopoiesis.

Expression of this gene product in primary dendritic cells also strongly indicates a role for this protein in immune function and immune surveillance.

Similarly, the tissue distribution in tonsils and immune cells indicates that polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and/or treatment of a variety of immune system disorders. Expression of this gene product in tonsils indicates a role in the regulation of the proliferation, survival, differentiation, and/or activation of potentially all hematopoietic cell lineages, including blood stem cells. This gene product may be involved in the regulation of cytokine production, antigen presentation, or other processes that may also suggest a usefulness in the treatment of cancer (e.g., by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the gene or protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immune deficiency diseases such as AIDS, leukemia, rheumatoid arthritis, inflammatory bowel disease, sepsis, acne, and psoriasis. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell



types. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:15 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 3213 of SEQ ID NO:15, b is an integer of 15 to 3227, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:15, and where b is greater than or equal to a + 14.

## 15 FEATURES OF PROTEIN ENCODED BY GENE NO: 6

Preferred polypeptides of the invention comprise the following amino acid sequence:

MLVYLITGDVKFGLLARVGCCLTVPTERCFFSFCAA VKKPAPAPPKPGNPPPG  
 20 HPGGQSSSGTSQHPPSLSPKPPTRSPSPPTQHTGQPPGQPSAPSQLSAPRRYSSS  
 LSPIQAPNHPPPQPPTQATPLMHTKPNSQGPPNPMALPSEHGLEQPSHTPPQTP  
 TPPSTPPLGKQNPSLPAPQTLAGGNPETAQPHAGTLPRPRVPKPRNRPSVPPP  
 PQPPGVHSAGDSSLTNTAPTASKIVTDV (SEQ ID NO: 193). Polynucleotides  
 encoding such polypeptides are also provided.

25 This gene is expressed primarily in human adult heart and to a lesser extent in a variety of highly vascularized tissues including colon carcinoma, placenta, rejected

kidney, normal colon, bone marrow, spleen, whole 8 week old embryo and fetal/liver spleen.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, cancer and other proliferative disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the heart and highly vascularized tissue, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder. Preferred polypeptides of the present invention comprise immunogenic epitopes shown in SEQ ID NO: 92 as residues: His-35 to Ala-40, Cys-62 to Glu-69, Pro-85 to Gly-96, Arg-111 to His-120. Polynucleotides encoding said polypeptides are also provided.

The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for diagnosis and treatment of cancer and other proliferative disorders.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:16 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is

cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1640 of SEQ ID NO:16, b is an integer of 15 to 1654, where both a and b correspond to the positions of nucleotide  
5 residues shown in SEQ ID NO:16, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 7**

10           The translation product of this gene shares sequence homology with rhodopsin and other proteins necessary for color vision. Accordingly, the translation product of this gene is believed to possess similar activity.

          This gene is expressed primarily in placenta and to a lesser extent in pregnant uterus and other tissue and cell types.

15           Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, color blindness. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential  
20 identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the eye, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having  
25 such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise immunogenic epitopes shown in SEQ ID NO: 93 as residues: Arg-75 to Lys-81, Gln-99 to Asp-109.

Polynucleotides encoding said polypeptides are also provided.

The tissue distribution and homology to rhodopsin indicates that  
5 polynucleotides and polypeptides corresponding to this gene are useful for treating ocular disorders.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:17 and may have been publicly available prior to conception of  
10 the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1749 of SEQ ID NO:17, b is an  
15 integer of 15 to 1763, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:17, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 8**

20

The gene encoding the disclosed cDNA is believed to reside on chromosome 10. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 10.

This gene is expressed primarily in the retina, activated T-cells, neutrophils  
25 and keratinocytes, and to a lesser extent in immune cell types in general and in various brain compartments.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, immune system, neurological and eye disorders. Similarly,

5 polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune and nervous systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, neural, cancerous

10 and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder. Preferred polypeptides of the present invention comprise immunogenic epitopes

15 shown in SEQ ID NO: 94 as residues: Met-1 to Phe-11, Thr-33 to Cys-40, Arg-42 to Arg-64. Polynucleotides encoding said polypeptides are also provided.

The tissue distribution of this gene predominantly in activated T-cells indicates that the gene could be important for the treatment and/or detection of immune or hematopoietic disorders including arthritis, asthma, immunodeficiency

20 diseases and leukemia. This gene product may be involved in the regulation of cytokine production, antigen presentation, or other processes that may also suggest a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the gene or protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or

25 immunotherapy targets for the above listed tissues. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immune deficiency

diseases such as AIDS, leukemia, rheumatoid arthritis, inflammatory bowel disease, sepsis, acne, and psoriasis. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types.

5           Alternatively, expression in the retina indicates a role in the detection or treatment of eye defects including impaired vision, blindness, cataracts, color blindness, short and long sightedness, retinitis pigmentosa, retinitis proliferans, retinoblastoma, retinochoroiditis, retinopathy and retinoschisis. Additionally, expression in the brain indicates a role in the detection and/or treatment of  
10   neurodegenerative disease states and behavioral disorders such as Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder and panic disorder. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

15           Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:18 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is  
20   cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 689 of SEQ ID NO:18, b is an integer of 15 to 703, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:18, and where b is greater than or equal to a + 14.

**FEATURES OF PROTEIN ENCODED BY GENE NO: 9**

This gene matches UniGene cluster Hs.69319 (match is to accession AA099388), which indicates that this gene maps to human chromosome 2 (stSG31094, Chr.2, D2S292-S2S145 according to Gene Map 98). Accordingly, polynucleotides of the invention are useful as chromosome markers in linkage analysis for chromosome 2.

This gene is expressed primarily in normal stomach.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, cancer and other proliferative disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the stomach, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder. Preferred polypeptides of the present invention comprise immunogenic epitopes shown in SEQ ID NO: 95 as residues: Asn-27 to Gly-36, Val-51 to Trp-60, Ile-63 to Asn-68, Lys-104 to Pro-116, Asn-124 to Asp-130. Polynucleotides encoding said polypeptides are also provided.

The tissue distribution indicates that this gene is useful for diagnosing and treating gastrointestinal ailments.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:19 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 760 of SEQ ID NO:19, b is an integer of 15 to 774, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:19, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 10

Preferred polypeptides of the invention comprise the following amino acid sequence:

FIFSVKKKKTDDGPSLGAQDQRSTPTNQKGSIIIPNNIRHKFGSNVVDQLVSEE  
QAQKAIDEVFEGQKRASSWPSRTQNPVEISSVFSDYYDLGYNMRSNLFGRGAA  
EETKSLMKASYTPEVIEKSVRDLEHWHGRKTDDLGRWHQKNAMNLNLQKA  
LEEKYGENSKSKSSKY (SEQ ID NO: 194) ,  
GSIIPNNIRHKFGSNVVDQLVSEEAQKAID (SEQ ID NO: 195),  
EVFEGQKRASSWPSRTQNPVEISSVFSDYYDLG (SEQ ID NO: 196)  
YNMRSNLFGRGAAEETKSLMKASYTPEVIEKSVRDLEHWHG (SEQ ID NO:  
197), RKTDDLGRWHQKNAMNLNLQKALEEKYGENSKSKSSKY (SEQ ID NO:  
198). Polynucleotides encoding these polypeptides are also encompassed by the invention.



The polypeptide of this gene has been determined to have a transmembrane domain at about amino acid position 162-178 of the amino acid sequence referenced in Table 1 for this gene. Moreover, a cytoplasmic tail encompassing amino acids 179-231 of this protein has also been determined. Based upon these characteristics, it is believed that the protein product of this gene shares structural features to type Ia membrane proteins.

The gene encoding the disclosed cDNA is believed to reside on chromosome 22. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 22.

10 This gene is expressed primarily in testes.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, male reproductive defects, sexual dysfunction, and/or infertility.

15 Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the male reproductive system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., testicular, cancerous and wounded tissues) or bodily fluids (e.g., lymph, semen, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder. Preferred polypeptides of the present invention comprise immunogenic  
25 epitopes shown in SEQ ID NO: 96 as residues: Gly-16 to Leu-23, Gly-31 to Glu-37, Thr-72 to Gly-77, Asn-83 to Glu-88, Lys-96 to Phe-109, Arg-117 to Gln-122, Arg-

183 to Ser-188, Asn-209 to Phe-215, Leu-218 to Ser-227. Polynucleotides encoding said polypeptides are also provided.

The tissue distribution in testes indicates that polynucleotides and polypeptides corresponding to this gene are useful for study and treatment of male reproductive disorders. Additionally, polynucleotides and polypeptides corresponding to this gene are useful for the treatment and diagnosis of conditions concerning proper testicular function (e.g. endocrine function, sperm maturation), as well as cancer. Therefore, this gene product is useful in the treatment of male infertility and/or impotence. This gene product is also useful in assays designed to identify binding agents, as such agents (antagonists) are useful as male contraceptive agents. Similarly, the protein is believed to be useful in the treatment and/or diagnosis of testicular cancer. The testes are also a site of active gene expression of transcripts that may be expressed, particularly at low levels, in other tissues of the body. Therefore, this gene product may be expressed in other specific tissues or organs where it may play related functional roles in other processes, such as hematopoiesis, inflammation, bone formation, and kidney function, to name a few possible target indications. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:20 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1535 of SEQ ID NO:20, b is an

integer of 15 to 1549, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:20, and where b is greater than or equal to a + 14.

## 5 FEATURES OF PROTEIN ENCODED BY GENE NO: 11

Preferred polypeptides of the invention comprise the following amino acid sequence: HESARGRWEGGRRACRGLGLARAQGAERVTSSEQRPA (SEQ ID NO: 199),

- 10 SQVPKRTDSSEPCGLSDLCRSLMTKPGCSGYCLSHQLLFFLWARMRGCTQGP  
LQQSQDYITFCANMMDLNRRAEAIGYAYPTRDIFMENIMFCGMGGFSDFYKL  
RWLEAILSWQKQKEGCFGEFDAEDEELSKAIQYQQHFSRRVKRREKQFPEYW  
KWCP (SEQ ID NO: 200),  
SQVPKRTDSSEPCGLSDLCRSLMTKPGCSGYCLSHQLLF (SEQ ID NO: 201),  
15 FLWARMRGCTQGPLQQSQDYITFCANMMDLNRRAEA (SEQ ID NO: 202),  
IGYAYPTRDIFMENIMFCGMGGFSDFYKL RWLEAILSWQKQKEG (SEQ ID  
NO: 203), and/or CFGEFDAEDEELSKAIQYQQHFSRRVKRREKQFPEYWKWCP  
(SEQ ID NO: 204). Polynucleotides encoding these polypeptides are also  
encompassed by the invention.

- 20 This gene is expressed primarily in ovarian tumor, fetal liver, activated T-cell,  
osteoblasts, leukocytes, tongue tumor, and bone cancer tissues and/or cells.

- Therefore, polynucleotides and polypeptides of the invention are useful as  
reagents for differential identification of the tissue(s) or cell type(s) present in a  
biological sample and for diagnosis of diseases and conditions which include, but are  
25 not limited to, ovarian tumors, tongue tumors, bone cancer, and immune disorders.  
Similarly, polypeptides and antibodies directed to these polypeptides are useful in

providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the hematopoietic and immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., skeletal, reproductive, hematopoietic, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder. Preferred polypeptides of the present invention comprise immunogenic epitopes shown in SEQ ID NO: 97 as residues: Gln-71 to Ala-80, Lys-111 to Gly-120. Polynucleotides encoding said polypeptides are also provided.

The tissue distribution in ovarian tumor, tongue tumor, bone cancer, fetal liver indicates that polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and treatment of cancer and other proliferative disorders. Expression within embryonic tissue and other cellular sources marked by proliferating cells indicates that this protein may play a role in the regulation of cellular division. Additionally, the expression in hematopoietic cells and tissues indicates that this protein may play a role in the proliferation, differentiation, and/or survival of hematopoietic cell lineages. In such an event, this gene may be useful in the treatment of lymphoproliferative disorders, and in the maintenance and differentiation of various hematopoietic lineages from early hematopoietic stem and committed progenitor cells. Similarly, embryonic development also involves decisions involving cell differentiation and/or apoptosis in pattern formation. Thus, this protein may also be involved in apoptosis or tissue differentiation and could again be useful in cancer therapy. As the translation product of this gene is observed in cells and/or tissues of

cancerous origins, the translation product of this gene may be a good target for immunotherapy. The tissue distribution in tumors of tongue, ovary, and bone indicates that polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and intervention of these tumors, in addition to other tumors where expression has been indicated. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:21 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1175 of SEQ ID NO:21, b is an integer of 15 to 1189, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:21, and where b is greater than or equal to a + 14.

## **20 FEATURES OF PROTEIN ENCODED BY GENE NO: 12**

The gene encoding the disclosed cDNA is believed to reside on chromosome 14. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 14.

25 The translation product of this gene shares sequence homology with a non-adrenergic smooth muscle binding protein (See Genseq Acc. No. W61371), a

membrane spanning receptor capable of binding iodocyanopindolol (ICYP) under blockade of alpha , beta 1, beta 2 and beta 3 adrenergic receptors and serotonin 5-HT1A and 5-HT1B receptors.

Preferred polypeptides of the invention comprise the following amino acid  
5 sequence:

KSVGRSSPTRRYRAAVGETPAGAQQXQLRGREGRRRLGQPFPRGSTALR  
(SEQ ID NO: 205). Polynucleotides encoding these polypeptides are also encompassed by the invention.

The polypeptide of this gene has been determined to have transmembrane  
10 domains at about amino acid positions 242-258, 499-515, 412-428, 317-333, 514-590, 536-552, 339-355, 373-389, and 464-480 of the amino acid sequence referenced in Table 1 for this gene. Based upon these characteristics, it is believed that the protein product of this gene shares structural features to type IIIa membrane proteins.

This gene is expressed primarily in breast, activated monocytes, T-cells,  
15 placenta and infant brain. The gene is also expressed in a number of normal and cancerous tissues.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are  
20 not limited to, reproductive disorders or immune disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the hematopoietic, reproductive or immune systems, expression of this gene at significantly higher or lower levels  
25 may be routinely detected in certain tissues or cell types (e.g., reproductive, hematopoietic, immune, cancerous and wounded tissues) or bodily fluids (e.g., lymph,

amniotic, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder. Preferred polypeptides of the present invention  
5 comprise immunogenic epitopes shown in SEQ ID NO: 98 as residues: Pro-7 to Cys-12, Lys-48 to Tyr-62, Arg-182 to His-187, Leu-189 to Glu-196, Thr-211 to Gly-226, Leu-270 to Thr-275, Gly-278 to Gly-289, Pro-444 to Asn-449, Glu-453 to Lys-461, Gly-491 to Thr-496, Ser-525 to Trp-532. Polynucleotides encoding said polypeptides are also provided.

- 10           The homology to a membrane receptor and its tissue distribution in a number of embryonic tissue and other cellular sources marked by proliferating cells indicates that this protein may play a role in the regulation of cellular division. Additionally, the expression in hematopoietic cells and tissues indicates that this protein may play a role in the proliferation, differentiation, and/or survival of hematopoietic cell lineages.
- 15   In such an event, this gene may be useful in the treatment of lymphoproliferative disorders, and in the maintenance and differentiation of various hematopoietic lineages from early hematopoietic stem and committed progenitor cells. Similarly, embryonic development also involves decisions involving cell differentiation and/or apoptosis in pattern formation. Thus this protein may also be involved in apoptosis or
- 20   tissue differentiation and could again be useful in cancer therapy. Elevated levels of expression of this gene product in T cell lineages indicates that it may play an active role in normal T cell function and in the regulation of the immune response. For example, this gene product may be involved in T cell activation, in the activation or control of differentiation of other hematopoietic cell lineages, in antigen recognition,
- 25   or in T cell proliferation. Similarly, the tissue distribution in monocytes and T-cells indicates that the polypeptides or polynucleotides are useful for treatment,

prophylaxis, and diagnosis of immune and autoimmune diseases, such as lupus, transplant rejection, allergic reactions, arthritis, asthma, immunodeficiency diseases, leukemia, and AIDS. The expression observed in hematopoietic cells also indicates that the polynucleotides or polypeptides are important in treating and/or detecting

5 hematopoietic disorders, such as graft versus host reaction, graft versus host disease, transplant rejection, myelogenous leukemia, bone marrow fibrosis, and myeloproliferative disease. The polypeptides or polynucleotides are also useful to enhance or protect proliferation, differentiation, and functional activation of hematopoietic progenitor cells (e.g., bone marrow cells), useful in treating cancer

10 patients undergoing chemotherapy or patients undergoing bone marrow transplantation. The polypeptides or polynucleotides are also useful to increase the proliferation of peripheral blood leukocytes, which can be used in the combat of a range of hematopoietic disorders, including immunodeficiency diseases, leukemia, and septicemia. Protein, as well as, antibodies directed against the protein may show

15 utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:22 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically

20 excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2446 of SEQ ID NO:22, b is an integer of 15 to 2460, where both a and b correspond to the positions of nucleotide

25 residues shown in SEQ ID NO:22, and where b is greater than or equal to a + 14.



**FEATURES OF PROTEIN ENCODED BY GENE NO: 13**

The translation product of this gene shares sequence homology with murine  
5 junctional adhesion molecule (JAM), which is thought to be important in promoting  
cell-to-cell homotypic adhesion. The gene encoding the disclosed cDNA is thought to  
reside on chromosome 11. Accordingly, polynucleotides related to this invention are  
useful as a marker in linkage analysis for chromosome 11.

The polypeptide of this gene has been determined to have a transmembrane  
10 domain at about amino acid position 249-265 of the amino acid sequence referenced  
in Table 1 for this gene. Moreover, a cytoplasmic tail encompassing amino acids 266-  
310 of this protein has also been determined. Based upon these characteristics, it is  
believed that the protein product of this gene shares structural features to type Ia  
membrane proteins.

15 This gene is expressed primarily in endothelial cells and fetal heart tissue, and  
to a lesser extent in fetal liver/spleen tissue.

Therefore, polynucleotides and polypeptides of the invention are useful as  
reagents for differential identification of the tissue(s) or cell type(s) present in a  
biological sample and for diagnosis of diseases and conditions which include, but are  
20 not limited to, inflammatory disorders, vascular disorders, and immune disorders.  
Similarly, polypeptides and antibodies directed to these polypeptides are useful in  
providing immunological probes for differential identification of the tissue(s) or cell  
type(s). For a number of disorders of the above tissues or cells, particularly of the  
vascular and immune systems, expression of this gene at significantly higher or lower  
25 levels may be routinely detected in certain tissues or cell types (e.g., vascular,  
immune, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum,

plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder. Preferred polypeptides of the present invention comprise

5 immunogenic epitopes shown in SEQ ID NO: 99 as residues: Leu-3 to Arg-8, Asp-57 to Arg-64, Glu-66 to Thr-75, Arg-120 to Ile-126, Gln-161 to Asp-177, Thr-182 to Ser-194, Lys-211 to Gln-216, Asn-274 to Gly-290, Thr-296 to Phe-302.

Polynucleotides encoding said polypeptides are also provided.

The tissue distribution in vascular and immune tissues, and the homology to  
10 the murine JAM protein, indicates that polynucleotides and polypeptides corresponding to this gene are useful for the treatment of vascular and immune disorders. Furthermore, the translation product of this gene is useful for the detection and/or treatment of acute and chronic inflammatory diseases, organ transplantation, myocardial ischemia, atherosclerosis, cancer, diabetic retinopathy, psoriasis, and  
15 rheumatoid arthritis. Additionally, the tissue distribution in fetal heart tissue indicates that the protein product of this gene is useful for the diagnosis and treatment of conditions and pathologies of the cardiovascular system, such as heart disease, restenosis, atherosclerosis, stroke, angina, thrombosis, and wound healing.

Expression of this gene product in immune cells and tissues indicates a role in  
20 the regulation of the proliferation; survival; differentiation; and/or activation of potentially all hematopoietic cell lineages, including blood stem cells. This gene product may be involved in the regulation of cytokine production, antigen presentation, or other processes that may also suggest a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of  
25 lymphoid origin, the gene or protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the

above listed tissues. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immune deficiency diseases such as AIDS, leukemia, rheumatoid arthritis, inflammatory bowel disease, sepsis, acne, and psoriasis. In addition, this gene product may have commercial utility in the expansion  
5 of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly  
10 available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:23 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or  
15 more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 4372 of SEQ ID NO:23, b is an integer of 15 to 4386, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:23, and where b is greater than or equal to a + 14.

20

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 14**

The translation product of this gene shares sequence homology with the C. elegans gene: yk27d2.3; coded for by C. elegans cDNA yk27d2.5; similar to C.  
25 elegans protein C40H1.5 in exon 1 [Caenorhabditis elegans] >sp|Q19330|Q19330 COSMID F10G7. The gene encoding the disclosed cDNA is thought to reside on

chromosome 2. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 2.

The polypeptide of this gene has been determined to have transmembrane domains at about amino acid positions 169-185 and 136-152 of the amino acid  
5 sequence referenced in Table 1 for this gene. Based upon these characteristics, it is believed that the protein product of this gene shares structural features to type IIIa membrane proteins.

This gene is expressed primarily in Germinal center B cells, fetal liver spleen, gall bladder, pregnant uterus, melanocyte and multiple sclerosis, and to a lesser extent  
10 in a variety of normal and transformed fetal and adult tissues.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, immune system disorders, cancer and other proliferative disorders.  
15 Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, cancerous and  
20 wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder. Preferred polypeptides of the present invention comprise immunogenic epitopes shown in SEQ  
25 ID NO: 100 as residues: Pro-29 to Glu-36, Phe-83 to Gly-91, Pro-110 to Thr-115,

Gly-202 to Lys-212, Phe-233 to Gly-246. Polynucleotides encoding said polypeptides are also provided.

The tissue distribution in immune cells and tissues indicates that polynucleotides and polypeptides corresponding to this gene are useful for the

5 diagnosis and/or treatment of cancer and other proliferative disorders. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections below, in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein. Expression of this gene product in immune system tissues and cells indicates a role in the regulation of the proliferation; survival; differentiation; and/or activation of potentially all

10 hematopoietic cell lineages, including blood stem cells. This gene product may be involved in the regulation of cytokine production, antigen presentation, or other processes that may also suggest a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the gene or protein, as well as, antibodies directed against the protein may show

15 utility as a tumor marker and/or immunotherapy targets for the above listed tissues. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immune deficiency diseases such as AIDS, leukemia, rheumatoid arthritis, inflammatory bowel disease, sepsis, acne, and psoriasis. In addition, this gene product may have commercial utility in the expansion of stem cells and

20 committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly

25 available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:24 and may have been publicly available prior to conception of

the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general  
5 formula of a-b, where a is any integer between 1 to 2448 of SEQ ID NO:24, b is an integer of 15 to 2462, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:24, and where b is greater than or equal to a + 14.

## 10 FEATURES OF PROTEIN ENCODED BY GENE NO: 15

Preferred polypeptides of the invention comprise the following amino acid sequence:

IFLFYLPPSPPSRLLVPGYWCLASWQGPGTWTISHTTPRGGIFFYFPYEKQIFLR

15 (SEQ ID NO: 206). Polynucleotides encoding these polypeptides are also encompassed by the invention.

The translation product of this gene shares homology with fragments of human fibronectin which inhibit binding of fibronectin or fibrinogen to fibronectin receptors and are useful for inhibiting platelet aggregation and coating the surface of  
20 prosthetic blood vessels or vascular grafts (e.g., See Genseq Acc. Nos. R08039, R60347 and/or R37614).

This gene is expressed primarily in fetal liver and heart tissues, and to a lesser extent in a variety of other tissues and cell types.

Therefore, polynucleotides and polypeptides of the invention are useful as  
25 reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are

not limited to, liver disorders and cancers (e.g., hepatoblastoma, hepatitis, liver metabolic diseases) and cardiovascular and respiratory or pulmonary disorders (e.g., asthma, pulmonary edema, pneumonia, atherosclerosis, restenosis, stroke, angina, thrombosis, hypertension, inflammation and wound healing). Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the cardiovascular and hepatic systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., liver, heart, cancerous and wounded tissues) or bodily fluids (e.g., lymph, bile, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder. Preferred polypeptides of the present invention comprise immunogenic epitopes shown in SEQ ID NO: 101 as residues: Ser-17 to Glu-28, Phe-57 to Arg-63, Glu-94 to Asp-106, Glu-108 to Gln-116, Tyr-128 to Val-133, Ser-152 to Thr-157, Thr-165 to Glu-175, Pro-258 to Phe-268, Ala-270 to Leu-276, Ser-282 to Asp-287, Arg-333 to Val-343, Leu-409 to Ala-415, Lys-422 to Gly-427, Arg-438 to Ser-443, Leu-447 to Arg-452, Thr-472 to Lys-477, Lys-483 to Leu-502, Asp-505 to Glu-511, Phe-518 to Leu-523. Polynucleotides encoding said polypeptides are also provided.

The tissue distribution in heart tissue, and the shared homology of the translation product of this gene to human fibronectin, indicates that polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis, detection and/or treatment of cardiovascular, respiratory and/or pulmonary disorders such as asthma, pulmonary edema, pneumonia, atherosclerosis, restenosis, stroke, angina, thrombosis hypertension, inflammation and wound healing. In addition, the

pronounced expression in liver tissue indicates a role in the diagnosis and/or treatment of liver disorders and cancers such as hepatoblastoma, jaundice, hepatitis, liver metabolic diseases and conditions that are attributable to the differentiation of hepatocyte progenitor cells. Protein, as well as, antibodies directed against the protein  
5 may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:25 and may have been publicly available prior to conception of  
10 the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2621 of SEQ ID NO:25, b is an  
15 integer of 15 to 2635, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:25, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 16**

20

This gene is expressed primarily in primary dendritic cells, macrophages and Rhabdosarcoma and to a lesser extent in a variety of other immune tissues such as monocytes, neutrophils, eosinophils, activated T-cells, CD34 depleted buffy coat (cord blood), T cell helpers, as well as in tissues which is expected to be contaminated  
25 with immune cells such as spleen from chronic lymphocytic leukemia, breast, fetal heart, L428, colon, osteoblasts, etc.



Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, cancer and other proliferative disorders, immunosuppressive disorders and autoimmune disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder. Preferred polypeptides of the present invention comprise immunogenic epitopes shown in SEQ ID NO: 102 as residues: Thr-52 to Phe-58. Polynucleotides encoding said polypeptides are also provided.

The tissue distribution in immune tissues indicates that polynucleotides and polypeptides corresponding to this gene are useful for diagnosis and treatment of cancer and other proliferative disorders, diseases involving immune suppression or hyperactivity, or other disease where immune modulation might be useful such as those listed elsewhere herein.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:26 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is

cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2693 of SEQ ID NO:26, b is an integer of 15 to 2707, where both a and b correspond to the positions of nucleotide  
5 residues shown in SEQ ID NO:26, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 17**

10           This gene is expressed primarily in testes tissue.

          Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, male reproductive defects and neoplasms. Similarly, polypeptides and  
15 antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the male reproductive system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or  
20 bodily fluids (e.g., semen, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder. Preferred polypeptides of the present invention comprise immunogenic epitopes shown in SEQ ID NO: 103 as  
25 residues: Pro-98 to Gln-106. Polynucleotides encoding said polypeptides are also provided.

The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for diagnosis, study and treatment of male reproductive and other development disorders and tumors.

Many polynucleotide sequences, such as EST sequences, are publicly  
5 available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:27 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or  
10 more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1884 of SEQ ID NO:27, b is an integer of 15 to 1898, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:27, and where b is greater than or equal to a + 14.

15

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 18**

The translation product of this gene shares sequence homology with a yeast gene which may be involved in modulating normal cell function.

20 When tested against K562 cell lines, supernatants removed from cells containing this gene activated the ISRE (interferon-sensitive responsive element ) promoter element. Thus, it is likely that this gene activates leukemia cells, and to a lesser extent, in immune and hematopoietic cells and tissues, through the JAK-STAT signal transduction pathway. ISRE is a promoter element found upstream in many  
25 genes which are involved in the Jak-STAT pathway. The Jak-STAT pathway is a large, signal transduction pathway involved in the differentiation and proliferation of

cells. Therefore, activation of the Jak-STAT pathway, reflected by the binding of the ISRE element, can be used to indicate proteins involved in the proliferation and differentiation of cells.

Preferred polypeptides of the invention comprise the following amino acid  
5 sequence:  
XRGMVFGGVVPYVPQYRDIRRTQNADGFSTYVCLVLLVANILRILFWFGRRF  
ESPLLWQSAI (SEQ ID NO: 207). Polynucleotides encoding these polypeptides are also encompassed by the invention.

The gene encoding the disclosed cDNA is believed to reside on chromosome  
10 18. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 18.

The polypeptide of this gene has been determined to have a transmembrane domain at about amino acid position 127-147 of the amino acid sequence referenced in Table 1 for this gene. Moreover, a cytoplasmic tail encompassing amino acids 1 -  
15 128 of this protein has also been determined. Based upon these characteristics, it is believed that the protein product of this gene shares structural features to type II membrane proteins.

This gene is expressed primarily in brain, fetal heart, and, to a lesser extent, in blood cells.

20 Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, neurodegenerative, cardiovascular, and developmental diseases and/or disorders. Similarly, polypeptides and antibodies directed to these polypeptides are  
25 useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells,

particularly of the central nervous system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., neurodegenerative, cardiovascular, muscular, and developmental cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, amniotic fluid, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder. Preferred polypeptides of the present invention comprise immunogenic epitopes shown in SEQ ID NO: 104 as residues: Ala-23 to His-34, His-153 to Ala-158.

10 Polynucleotides encoding said polypeptides are also provided.

The tissue distribution in brain tissue indicates that polynucleotides and polypeptides corresponding to this gene are useful for diagnosing or treating diseases of the central nervous system. Moreover, polynucleotides and polypeptides corresponding to this gene are useful for the detection, treatment, and/or prevention of neurodegenerative disease states, behavioral disorders, or inflammatory conditions which include, but are not limited to Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette Syndrome, meningitis, encephalitis, demyelinating diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, depression, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, elevated expression of this gene product in regions of the brain indicates it plays a role in normal neural function. Potentially, this gene product is involved in synapse formation, neurotransmission, learning, cognition, homeostasis, or neuronal differentiation or survival. In addition, the expression within fetal tissue, combined

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with the detected ISRE biological activity indicates this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis, treatment, and/or prevention of developmental diseases and disorders, cancer, and other proliferative conditions. Similarly, developmental tissues rely on decisions involving cell differentiation and/or apoptosis in pattern formation. Dysregulation of apoptosis can result in inappropriate suppression of cell death, as occurs in the development of some cancers, or in failure to control the extent of cell death, as is believed to occur in acquired immunodeficiency and certain neurodegenerative disorders, such as spinal muscular atrophy (SMA). Therefore, the polynucleotides and polypeptides of the present invention are useful in treating, detecting, and/or preventing said disorders and conditions, in addition to other types of degenerative conditions. Thus this protein may modulate apoptosis or tissue differentiation and is useful in the detection, treatment, and/or prevention of degenerative or proliferative conditions and diseases. The protein is useful in modulating the immune response to aberrant polypeptides, as may exist in proliferating and cancerous cells and tissues. The protein can also be used to gain new insight into the regulation of cellular growth and proliferation. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:28 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2284 of SEQ ID NO:28, b is an

integer of 15 to 2298, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:28, and where b is greater than or equal to a + 14.

## 5 FEATURES OF PROTEIN ENCODED BY GENE NO: 19

The translation product of this gene shows homology to several human and murine proteins with histidine rich charge clusters (e.g, See Genbank Acc. Nos. gnllPIDle1339015 (AL031228) dJ1033B10.10 and gil3811387 (AF100956)).

10 Preferred polypeptides of the invention comprise the following amino acid sequence:

RTGWLGPSPPPPHV RGMPCPCPGCMAGPRLLFLXALALELLGRAGGS  
QPALRSRGTTACRLDNKESESWGALLSGERLDTWICSLLGSLMVGLSGVFP  
LLVIPLEMGTMLRSEAGAWRLKQLLSFALGGLLGNVFLHLLPEAWAYTCSAS  
15 PGGEGQSLQQQQQLGLWVIAGILTF LALEKMF LDSKEEGTSQAPNKDPTAAA  
AALNGGHCLAQPAAEPGLGAVVRSIKVSGYLNLLANTIDNFTHGLAVAASFL  
VSKKIGLLTTMAILLHEIPHEVGDFAILLRAGFDRWSAAKLQLSTALGGLLGA  
GFAICTQSPKGVEETAAWVLPFTSGGFLYIALVNVLPDLLEEEDPWRSLQQLL  
LLCAGIVVMVLFSLFVD (SEQ ID NO: 208). Polynucleotides encoding these

20 polypeptides are also encompassed by the invention.

The polypeptide of this gene has been determined to have transmembrane domains at about amino acid positions 337-353 and 63-79 of the amino acid sequence referenced in Table 1 for this gene. Based upon these characteristics, it is believed that the protein product of this gene shares structural features to type IIIa membrane  
25 proteins.

This gene is expressed primarily in testis, cerebellum, dendritic cells, pharynx, breast and to a lesser extent in some other normal and transformed cell types.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of reproductive, neurological and/or immune diseases or disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the CNS and male reproductive organs, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., testes, CNS, cancerous and wounded tissues) or bodily fluids (e.g., lymph, semen, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in testes and cerebellum indicates that polynucleotides and polypeptides corresponding to this gene are useful for study and treatment of disorders of fertility and reproduction, and immune and nervous system development and function. Polynucleotides and polypeptides corresponding to this gene are useful for the treatment and diagnosis of conditions concerning proper testicular function (e.g. endocrine function, sperm maturation), as well as cancer. Therefore, this gene product is useful in the treatment of male infertility and/or impotence. This gene product is also useful in assays designed to identify binding agents, as such agents (antagonists) are useful as male contraceptive agents. Similarly, the protein is believed to be useful in the treatment and/or diagnosis of testicular cancer. The testes are also a site of active gene expression of transcripts that may be expressed,



particularly at low levels, in other tissues of the body. Therefore, this gene product may be expressed in other specific tissues or organs where it may play related functional roles in other processes, such as hematopoiesis, inflammation, bone formation, and kidney function, to name a few possible target indications.

- 5 Additionally, polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and/or treatment of disorders of the brain and nervous system. It may also be useful in the treatment of such neurodegenerative disorders as schizophrenia; ALS; or Alzheimer's. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the
- 10 above listed tissues.

- Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:29 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically
- 15 excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1467 of SEQ ID NO:29, b is an integer of 15 to 1481, where both a and b correspond to the positions of nucleotide
- 20 residues shown in SEQ ID NO:29, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 20**

- 25 Preferred polypeptides of the invention comprise the following amino acid sequence: RVRKWERSQPRLLYTGKLSGPQAR (SEQ ID NO: 209),

SPAWAQLPQSHPLPTASGLKNIPGIRGALTTRPSESPPAWNLAISNLLPSASWI  
 KLETAGTPGMSLPILPCLCSFLDLTYFFCFCFHPSCCLSCPEG (SEQ ID NO:  
 210), RPSESPPAWNLAISNLLPSASWIKLETAGTPGMSLP (SEQ ID NO: 211),  
 ILPCLCSFLDLTYFFCFCFHPSCCLSCPEG (SEQ ID NO: 212),

5 MGRDIPGVPAVSSLIQEALGRRLLMARFQAGGDSEGRVVNAPLIPGIFFRPEA  
 VGRGWLCGSAQAGLQNHPLWGDDGGQFQGPPAIHWAVWLRLSAVATEA  
 LSQATDAKDGQDDQEDDDPHGAREELVLLAAAVTTAFESFGAGKDETF  
 GCNLLGASQQAEQQGGREAGDPSLGHPGLGATELSCVEKAGLRPLPLPDA  
 (SEQ ID NO: 213). Polynucleotides encoding these polypeptides are also  
 10 encompassed by the invention.

The polypeptide of this gene has been determined to have a transmembrane  
 domain at about amino acid position 58-74 of the amino acid sequence referenced in  
 Table 1 for this gene. Moreover, a cytoplasmic tail encompassing amino acids 75-145  
 of this protein has also been determined. Based upon these characteristics, it is  
 15 believed that the protein product of this gene shares structural features to type Ia  
 membrane proteins.

This gene is expressed primarily in adult small intestine, and to a lesser extent,  
 in osteoarthritis; fraction I, and gall bladder.

Therefore, polynucleotides and polypeptides of the invention are useful as  
 20 reagents for differential identification of the tissue(s) or cell type(s) present in a  
 biological sample and for diagnosis of gastro-intestinal disorders and diseases.  
 Similarly, polypeptides and antibodies directed to these polypeptides are useful in  
 providing immunological probes for differential identification of the tissue(s) or cell  
 type(s). For a number of disorders of the above tissues or cells, particularly of the  
 25 digestive system, expression of this gene at significantly higher or lower levels may  
 be routinely detected in certain tissues or cell types (e.g., gastro-intestinal, cancerous

and wounded tissues) or bodily fluids (e.g., lymph, bile, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder. Preferred polypeptides of the present invention comprise immunogenic epitopes shown in SEQ ID NO: 106 as residues: Ser-44 to Leu-51, Arg-81 to Cys-94, Thr-118 to Tyr-126, Arg-129 to Ile-140. Polynucleotides encoding said polypeptides are also provided.

The tissue distribution in adult small intestine and gall bladder indicates that polynucleotides and polypeptides corresponding to this gene are useful for diagnosis, prevention, and/or treatment of gastro-intestinal disorders, for example diverticulitis or various metabolic disorders such as Tay-Sachs disease, phenylketonuria, galactosemia, porphyrias, and Hurler's syndrome. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:30 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 998 of SEQ ID NO:30, b is an integer of 15 to 1012, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:30, and where b is greater than or equal to a + 14.

**FEATURES OF PROTEIN ENCODED BY GENE NO: 21**

Preferred polypeptides of the invention comprise the following amino acid  
5 sequence: ARAARGKIESNLI (SEQ ID NO: 214). Polynucleotides encoding these  
polypeptides are also encompassed by the invention.

This gene is expressed primarily in fetal liver/spleen and tonsil tissues, and to  
a lesser extent in breast cancer and brain tissues.

Therefore, polynucleotides and polypeptides of the invention are useful as  
10 reagents for differential identification of the tissue(s) or cell type(s) present in a  
biological sample and for diagnosis of diseases and conditions which include, but are  
not limited to, hematopoietic or immune disorders. Similarly, polypeptides and  
antibodies directed to these polypeptides are useful in providing immunological  
probes for differential identification of the tissue(s) or cell type(s). For a number of  
15 disorders of the above tissues or cells, particularly of the hematopoietic and immune  
systems, expression of this gene at significantly higher or lower levels may be  
routinely detected in certain tissues or cell types (e.g., hematopoietic, cancerous and  
wounded tissues) or bodily fluids (e.g., lymph, amniotic fluid, serum, plasma, urine,  
synovial fluid and spinal fluid) or another tissue or cell sample taken from an  
20 individual having such a disorder, relative to the standard gene expression level, i.e.,  
the expression level in healthy tissue or bodily fluid from an individual not having the  
disorder. Preferred polypeptides of the present invention comprise immunogenic  
epitopes shown in SEQ ID NO: 107 as residues: Asn-36 to Gln-41, Pro-49 to Ser-54,  
Cys-65 to Ser-70. Polynucleotides encoding said polypeptides are also provided.

25 The tissue distribution in fetal liver and/or spleen and in tonsil tissues  
indicates that polynucleotides and polypeptides corresponding to this gene are useful

for the diagnosis and/or treatment of hematopoietic and/or immune disorders.

Expression of this gene product in tonsils indicates a role in the regulation of the proliferation, survival, differentiation, and/or activation of potentially all hematopoietic cell lineages, including blood stem cells. In such an event, this gene

- 5 may be useful in the treatment of lymphoproliferative disorders, and in the maintenance and differentiation of various hematopoietic lineages from early hematopoietic stem and committed progenitor cells. The tissue distribution indicates that the polypeptides or polynucleotides are useful for treatment, prophylaxis, and diagnosis of immune and autoimmune diseases, such as lupus, transplant rejection,
- 10 allergic reactions, arthritis, asthma, immunodeficiency diseases, leukemia, and AIDS. The polypeptides or polynucleotides of the present invention are also useful in the treatment, prophylaxis, and detection of thymus disorders, such as Grave's Disease, lymphocytic thyroiditis, hyperthyroidism, and hypothyroidism. The expression observed predominantly in hematopoietic cells also indicates that the polynucleotides
- 15 or polypeptides are important in treating and/or detecting hematopoietic disorders, such as graft versus host reaction, graft versus host disease, transplant rejection, myelogenous leukemia, bone marrow fibrosis, and myeloproliferative disease. The polypeptides or polynucleotides are also useful to enhance or protect proliferation, differentiation, and functional activation of hematopoietic progenitor cells (e.g., bone
- 20 marrow cells), useful in treating cancer patients undergoing chemotherapy or patients undergoing bone marrow transplantation. The polypeptides or polynucleotides are also useful to increase the proliferation of peripheral blood leukocytes, which can be used in the combat of a range of hematopoietic disorders, including immunodeficiency diseases, leukemia, and septicemia. Additionally,
- 25 polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and treatment of cancer and other proliferative disorders. Expression within

embryonic tissue and other cellular sources marked by proliferating cells indicates that this protein may play a role in the regulation of cellular division. Similarly, embryonic development also involves decisions involving cell differentiation and/or apoptosis in pattern formation. Thus this protein may also be involved in apoptosis or tissue differentiation and could again be useful in cancer therapy. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:31 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1872 of SEQ ID NO:31, b is an integer of 15 to 1886, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:31, and where b is greater than or equal to a + 14.

## **20 FEATURES OF PROTEIN ENCODED BY GENE NO: 22**

Preferred polypeptides of the invention comprise the following amino acid sequence:

HMLWNRRLRCCFHKFVLSLALGPSFLFWKNLSEKRDLSVCSAFLYKTRNG  
VNSRDMEVITPDSLCLWLLRFSQGEV (SEQ ID NO: 216), and/or GPQVDWQRPL  
(SEQ ID NO: 215). Polynucleotides encoding these polypeptides are also

encompassed by the invention. On the nucleotide level, this gene shares sequence homology with 14-3-3 protein gamma-subtype mRNA (e.g., See Genbank Acc. No. gb|D17447|D17447 and/or gb|AF058799|AF058799). The 14-3-3 protein is a putative regulatory protein for protein kinase C and may be involved in the elaborate

5 regulation of some fundamental cellular activities and differentiation of neurons.

This gene is expressed primarily in pregnant uterus, placenta, fetal brain, osteoclastoma and to a lesser extent in a variety of tumor tissues.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a  
10 biological sample and for diagnosis of diseases and conditions which include, but are not limited to, reproductive, developmental disorders, and neurological disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the  
15 CNS, or reproductive system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., placenta, fetal, neural, cancerous and wounded tissues) or bodily fluids (e.g., lymph, amniotic fluid, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene  
20 expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder. Preferred polypeptides of the present invention comprise immunogenic epitopes shown in SEQ ID NO: 108 as residues: Ser-15 to Thr-31. Polynucleotides encoding said polypeptides are also provided.

Expression within embryonic tissue and other cellular sources marked by  
25 proliferating cells, and the shared homology with the 14-3-3 family of proteins involved in regulation of protein kinase C, indicates that this protein may play a role

in the regulation of cellular division, and may show utility in the diagnosis and treatment of cancer and other proliferative disorders. Similarly, embryonic development also involves decisions involving cell differentiation and/or apoptosis in pattern formation. Thus, this protein may also be involved in apoptosis or tissue differentiation and could again be useful in cancer therapy. Similarly, the tissue distribution in pregnant uterus and placenta indicates that polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and/or treatment of disorders of the placenta. Specific expression within the placenta indicates that this gene product may play a role in the proper establishment and maintenance of placental function. Alternatively, this gene product may be produced by the placenta and then transported to the embryo, where it may play a crucial role in the development and/or survival of the developing embryo or fetus. Expression of this gene product in a vascular-rich tissue such as the placenta also indicates that this gene product may be produced more generally in endothelial cells or within the circulation. In such instances, it may play more generalized roles in vascular function, such as in angiogenesis. It may also be produced in the vasculature and have effects on other cells within the circulation, such as hematopoietic cells. It may serve to promote the proliferation, survival, activation, and/or differentiation of hematopoietic cells, as well as other cells throughout the body. Alternatively, the tissue distribution in osteoclastoma indicates that polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and/or treatment of bone and hematopoietic disorders. Elevated levels of expression of this gene product in osteoclastoma indicates that it may play a role in the survival, proliferation, and/or growth of osteoclasts. Therefore, it may be useful in influencing bone mass in such conditions as osteoporosis. More generally, as evidenced by expression in fetal liver/spleen, this gene may play a role in the survival, proliferation, and/or differentiation of



hematopoietic cells in general, and may be of use in augmentation of the numbers of stem cells and committed progenitors. Expression of this gene product in primary dendritic cells also indicates that it may play a role in mediating responses to infection and controlling immunological responses, such as those that occur during immune surveillance. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:32 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2392 of SEQ ID NO:32, b is an integer of 15 to 2406, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:32, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 23**

Preferred polypeptides of the invention comprise the following amino acid sequence:

MVTRAGAGTAVAGAVVVALLSAALALYGPPLDAVLERAFLRKAHSIKDME  
NTLQLVRNIIPPLSSTKHKGQDGRIGVVGGCQEYTGAPYFARISALKVGADLS  
HVFCASAAAPVIKAYSPELIVHPVLDSNAVHEVEKWLPRLHALVVGPGGLGR  
DDALLRNVQGILEVSKARDIPVVIDADGLWXVAQQPALIHGYRKAVLTPNHV

EFSRLYDAVLRGPMDSDDSHGSVLRLSQALGNVTVVQKGERDILSNGQQVL  
VCSQEGSSAGVEGKGTSCRAPWASW (SEQ ID NO: 217). Also preferred are  
polypeptide fragments of the foregoing sequence as specified elsewhere herein. Such  
fragments (including the below listed polypeptide epitopes) preferably do not

5 comprise the following polypeptide or any fragment thereof:

MAWVEMIVHPVLDSPLNAVHEVEKWLPRHALVVGTGLGRDDALLRNVQGI  
LEVSKARDIPVVIDADGLWLVAQQPALIHGYRKAVLTPNHVEFSRLYDAVLR  
GPMDSDDRCLVP (SEQ ID NO: 218). Polynucleotides encoding all of the  
foregoing polypeptides are also provided.

10 The gene encoding the disclosed cDNA is believed to reside on chromosome  
13. Accordingly, polynucleotides related to this invention are useful as a marker in  
linkage analysis for chromosome 13.

This gene is expressed primarily in Soares\_fetal\_heart\_NbHH19W and Soares  
adult brain N2b4HB55Y cDNA libraries and to a lesser extent in germinal center B  
15 cell, pooled human melanocyte, fetal heart, Soares\_pregnant\_uterus\_NbHPU and  
pregnant and Soares\_total\_fetus\_Nb2HF8\_9w cDNA libraries.

Therefore, polynucleotides and polypeptides of the invention are useful as  
reagents for differential identification of the tissue(s) or cell type(s) present in a  
biological sample and for diagnosis of diseases and conditions which include, but are  
20 not limited to, neurodegenerative and developmental disorders. Similarly,  
polypeptides and antibodies directed to these polypeptides are useful in providing  
immunological probes for differential identification of the tissue(s) or cell type(s). For  
a number of disorders of the above tissues or cells, particularly of the central nervous  
and fetal systems, expression of this gene at significantly higher or lower levels may  
25 be routinely detected in certain tissues or cell types (e.g., cancerous and wounded  
tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or

another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred immunogenic epitopes include those comprising a sequence shown  
5 in SEQ ID NO: 56 as residues: Ser-65 to Gly-74, Cys-82 to Gly-87, Ser-94 to Gln-101. Polynucleotides encoding such polypeptide epitopes are also encompassed by the invention.

Preferred polypeptides of the present invention comprise immunogenic  
epitopes shown in SEQ ID NO: 109 as residues: Ser-65 to Gly-74, Cys-82 to Gly-87,  
10 Ser-94 to Gln-101. Polynucleotides encoding said polypeptides are also provided.

The tissue distribution indicates that polynucleotides and polypeptides  
corresponding to this gene are useful for diagnosis and treatment of  
neurodegenerative disorders and/or developmental disorders such as those specified  
elsewhere herein.

15 Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:33 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is  
20 cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2622 of SEQ ID NO:33, b is an integer of 15 to 2636, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:33, and where b is greater than or equal to a + 14.

**FEATURES OF PROTEIN ENCODED BY GENE NO: 24**

Preferred polypeptides of the invention comprise the following amino acid sequence:

5 EFGTRLRAVASVGAALILFPCLLYGAYAFLPFDVPRPRLPTMSSRLIYTLRCGVF  
ATFPIVLGILVYGLSLLCFSALRPFGEPRREVEIHRRYVAQSVQLFILYFFNLAV  
LSTYLPQDTLKLPLLTGLFAVSRLIYWLTFVGRSFRGFGYGLTFLPLLSML  
MWNLYYMFVVEPERMLTATESRLDYPDHARSASDYRPRPWG (SEQ ID NO:  
219) or residues 1 - 55 of the forgoing sequence. Polynucleotides encoding such  
10 polypeptides are also encompassed by the invention.

This gene is expressed primarily in larynx and to a lesser extent in endometrial tumor and in neutrophils.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a  
15 biological sample and for diagnosis of diseases and conditions which include, but are not limited to, endometrial tumors and/or endometriosis and in cancer generally. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the  
20 female reproductive system and the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e.,  
25 the expression level in healthy tissue or bodily fluid from an individual not having the disorder. Preferred polypeptides of the present invention comprise immunogenic

epitopes shown in SEQ ID NO: 110 as residues: Pro-38 to Glu-45, Thr-141 to Asp-146, Pro-148 to Trp-162. Polynucleotides encoding said polypeptides are also provided.

The tissue distribution indicates that polynucleotides and polypeptides  
5 corresponding to this gene are useful for diagnosis and treatment of endometrial tumors, cancer and other immune system disorders.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:34 and may have been publicly available prior to conception of  
10 the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1447 of SEQ ID NO:34, b is an  
15 integer of 15 to 1461, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:34, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 25**

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The translation product of this gene shares sequence homology with yeast mannosyltransferase (See Genbank Accession Nos. gnlIPIDle228221 and gnlIPIDle1351618) which is thought to be important in glycosylation of protein in the endoplasmic reticulum.

25

The polypeptide of this gene has been determined to have a transmembrane domain at about amino acid position 18-34 of the amino acid sequence referenced in

Table 1 for this gene. Moreover, a cytoplasmic tail encompassing amino acids 35-258 of this protein has also been determined. Based upon these characteristics, it is believed that the protein product of this gene shares structural features to type Ib membrane proteins.

5           The gene encoding the disclosed cDNA is believed to reside on chromosome 11. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 11.

          This gene is expressed primarily in bone marrow, and to a lesser extent, in fetal liver and ulcerative colitis.

10           Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, immune and hematopoietic diseases and/or disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing

15 immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the hemapoietic system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, hematopoietic, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial

20 fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder. Preferred polypeptides of the present invention comprise immunogenic epitopes shown in SEQ ID NO: 111 as residues: Pro-12 to Phe-18, Ser-139 to Pro-146, Asp-

25 162 to Arg-173, Thr-188 to Glu-204, Lys-245 to Gly-258. Polynucleotides encoding said polypeptides are also provided.

The tissue distribution in bone marrow tissue, combined with the homology to the conserved mannosyltransferase protein indicates that polynucleotides and polypeptides corresponding to this gene are useful for diagnosis and treatment of disorders related to bone marrow functions such as hemapoiesis, anemia or leukemia.

- 5 The mannosyltransferase-like activity can be used for modulation or as a therapeutic target of cytokines, cell surface markers in hemapoiesis. The uses include bone marrow cell ex- vivo culture, bone marrow transplantation, bone marrow reconstitution, radiotherapy or chemotherapy of neoplasia. The gene product may also be involved in lymphopoiesis, therefore, it can be used in immune disorders such as
- 10 infection, inflammation, allergy, immunodeficiency etc. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed
- 15 tissues.

- Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:35 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically
- 20 excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 939 of SEQ ID NO:35, b is an integer of 15 to 953, where both a and b correspond to the positions of nucleotide
- 25 residues shown in SEQ ID NO:35, and where b is greater than or equal to a + 14.

**FEATURES OF PROTEIN ENCODED BY GENE NO: 26**

Preferred polypeptides of the invention comprise the following amino acid  
5 sequence: TWGHVHTTARAYCVSRWLVLCLR (SEQ ID NO: 220). Polynucleotides  
encoding these polypeptides are also encompassed by the invention.

The polypeptide of this gene has been determined to have a transmembrane  
domain at about amino acid position 25-41 of the amino acid sequence referenced in  
Table 1 for this gene. Moreover, a cytoplasmic tail encompassing amino acids 1 - 24  
10 of this protein has also been determined. Based upon these characteristics, it is  
believed that the protein product of this gene shares structural features to type II  
membrane proteins.

This gene is expressed primarily in soares\_fetal\_liver\_spleen\_INFLS\_S1 and  
stratagene pancreas (#937208) and to a lesser extent in fetal dura mater, fetal lung III,  
15 and activated T-cell (12hs)/thiouridine labeled eco.

Therefore, polynucleotides and polypeptides of the invention are useful as  
reagents for differential identification of the tissue(s) or cell type(s) present in a  
biological sample and for diagnosis of developmental disorders. Similarly,  
polypeptides and antibodies directed to these polypeptides are useful in providing  
20 immunological probes for differential identification of the tissue(s) or cell type(s). For  
a number of disorders of the above tissues or cells, particularly of the fetus, or  
reproductive system, expression of this gene at significantly higher or lower levels  
may be routinely detected in certain tissues or cell types (e.g., reproductive, fetal,  
cancerous and wounded tissues) or bodily fluids (e.g., lymph, amniotic, sputum,  
25 serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample  
taken from an individual having such a disorder, relative to the standard gene



expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder. Preferred polypeptides of the present invention comprise immunogenic epitopes shown in SEQ ID NO: 112 as residues: Ser-48 to Asp-57. Polynucleotides encoding said polypeptides are also provided.

5           The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for diagnosis and treatment of fetal developmental disorders. Expression within embryonic tissue and other cellular sources marked by proliferating cells indicates that this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis and treatment of  
10 cancer and other proliferative disorders. Similarly, embryonic development also involves decisions involving cell differentiation and/or apoptosis in pattern formation. Thus, this protein may also be involved in apoptosis or tissue differentiation and could again be useful in cancer therapy. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets  
15 for the above listed tissues.

          Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:36 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically  
20 excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1326 of SEQ ID NO:36, b is an integer of 15 to 1340, where both a and b correspond to the positions of nucleotide  
25 residues shown in SEQ ID NO:36, and where b is greater than or equal to a + 14.

**FEATURES OF PROTEIN ENCODED BY GENE NO: 27**

The translation product of this gene shares sequence homology with the C-terminal portion of human epidermal growth factor (EGF) module-containing seven transmembrane (7TM) receptors or surface molecules (e.g., See Genbank Acc. No. gil784994 and gnlIPIDle214264). Proteins with seven transmembrane segments (7TM) define a superfamily of receptors (7TM receptors) sharing the same topology: an extracellular N-terminus, three extramembranous loops on either side of the plasma membrane, and a cytoplasmic tail. Upon ligand binding, cytoplasmic portions of the activated receptor interact with heterotrimeric G-coupled proteins to induce various second messengers. A small group, recently recognized on the basis of homologous primary amino acid sequences, comprises receptors to hormones of the secretin/vasoactive intestinal peptide/glucagon family, parathyroid hormone and parathyroid hormone-related peptides, growth hormone-releasing factor, corticotropin-releasing factor, and calcitonin. The polynucleotide sequence of this gene may have a frame shift, therefore, preferred polypeptides of the invention comprise the following amino acid sequence:

GTSFSILSLAACLVVEAVVWKS SVTKNRTSY (SEQ ID NO: 221),  
 20 HWGLMLFYRLVFILHETSRSTQKAI AFCLGYGCPLAISVITLGATQPREVYTR  
 KNCVWLNWEDTKALLAFAIPALIIVVNITITIVVITKILRPSIGDKPCKQEKSS  
 LFQISKSIGVLTPLLGLTWGFGLTTVP GTNLVFHIIFAILNVFQGLFILLFGCL  
 WDLKVQEALLNKFSLSRWSSQHSKSTSLGS  
 STPVFSMSSPISRFRFNNLFGKTGTYNVSTPEATSSSLENSSSASSLLN (SEQ ID  
 25 NO: 222), HWGLMLFYRLVFILHETSRSTQKAI AFCLGYGCPLA (SEQ ID NO:  
 223), ISVITLGATQPREVYTRKNCVWLNWEDTKALLAFA (SEQ ID NO: 224),

IPALIIVVVNITITIVVITKILRPSIGDKPCKQEK (SEQ ID NO: 225),  
SSLFQISKSIGVLTPLLGLTWGFGGLTTVPFGTNLVF (SEQ ID NO: 226),  
HIIFAILNVFQGLFILLFGCLWDLKVQEALLNKFSL (SEQ ID NO: 227),  
SRWSSQHSKSTSLGSSTPVFSMSSPISRRFNNLFG (SEQ ID NO: 228), and/or

- 5 KTGTYNVSTPEATSSSLENSSSASSLLN (SEQ ID NO: 229). Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in human fetal brain, lymph node, and prostate and to a lesser extent in a wide range of tissues and organs.

- Therefore, polynucleotides and polypeptides of the invention are useful as
- 10 reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, neurological/psychological disorders, immunity related diseases and endocrine disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential
- 15 identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the neural, immune and endocrine systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., CNS, endocrine, immune, cancerous and wounded tissues) or bodily fluids (e.g., lymph, amniotic fluid, serum, plasma, urine, synovial fluid and
- 20 spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

- The tissue distribution in human fetal brain, lymph node, and prostate and homology to human epidermal growth factor (EGF) module-containing mucin-like
- 25 hormone receptor 1 (EMR1) 7TM family members indicates that polynucleotides and polypeptides corresponding to this gene are useful for the treatment and/or diagnosis

of neurological and/or psychological disorders, immunity related diseases and/or endocrine disorders. Polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and/or treatment of a variety of immune system disorders. This gene product may be involved in the regulation of cytokine production, antigen presentation, or other processes that may also suggest a usefulness in the treatment of cancer (e.g., by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the gene or protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immune deficiency diseases such as AIDS, leukemia, rheumatoid arthritis, inflammatory bowel disease, sepsis, acne, psoriasis, neutropenia, neutrophilia, tissue necrosis, neoplasia, granulomatous disease, systemic lupus erythematosus, drug induced hemolytic anemia, Sjogren's disease, scleroderma, hypersensitivities, such as T-cell mediated cytotoxicity, immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, infections, and other inflammatory diseases and complications. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types.

Alternatively, the tissue distribution in fetal brain indicates that polynucleotides and polypeptides corresponding to this gene are useful for the detection/treatment of neurodegenerative disease states and behavioral disorders such as Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette Syndrome, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, the

gene or gene product may also play a role in the treatment and/or detection of developmental disorders associated with the developing embryo, or sexually-linked disorders. In addition, the gene or gene product may also play a role in the diagnosis and/or treatment of disorders of the brain and nervous system (e.g., trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, toxic neuropathies induced by neurotoxins, inflammatory diseases such as meningitis and encephalitis, demyelinating diseases, neurodegenerative diseases such as peripheral neuropathies, multiple sclerosis, and neoplasia of neuroectodermal origin, etc.). The expression in prostate tissue may indicate the gene or its products can be used in the disorders of the prostate, including inflammatory disorders, such as chronic prostatitis, granulomatous prostatitis and malacoplakia, prostatic hyperplasia and prostate neoplastic disorders, including adenocarcinoma, transitional cell carcinomas, ductal carcinomas, squamous cell carcinomas, or as hormones or factors with systemic or reproductive functions. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:37 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2185 of SEQ ID NO:37, b is an integer of 15 to 2199, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:37, and where b is greater than or equal to a + 14.

**FEATURES OF PROTEIN ENCODED BY GENE NO: 28**

5 Preferred polypeptides of the invention comprise the following amino acid sequence: TRPLWIPRSLVLVE (SEQ ID NO: 230). Polynucleotides encoding these polypeptides are also encompassed by the invention.

The gene encoding the disclosed cDNA is believed to reside on chromosome 3. Accordingly, polynucleotides related to this invention are useful as a marker in  
10 linkage analysis for chromosome 3.

This gene is expressed primarily in liver, and to a lesser extent in testes tissue.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are  
15 not limited to, liver disorders and cancers (e.g., hepatoblastoma, liver metabolic diseases and conditions that are attributable to the differentiation of hepatocyte progenitor cells). Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above  
20 tissues or cells, particularly of the liver, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., liver, cancerous and wounded tissues) or bodily fluids (e.g., lymph, bile, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e.,  
25 the expression level in healthy tissue or bodily fluid from an individual not having the disorder. Preferred polypeptides of the present invention comprise immunogenic

epitopes shown in SEQ ID NO: 114 as residues: Pro-171 to Gln-179, Leu-218 to Lys-225, Phe-266 to Cys-275. Polynucleotides encoding said polypeptides are also provided.

The tissue distribution in liver indicates that polynucleotides and polypeptides  
5 corresponding to this gene are useful for the detection and treatment of liver disorders and cancers (e.g., hepatoblastoma, jaundice, hepatitis, liver metabolic diseases and conditions that are attributable to the differentiation of hepatocyte progenitor cells). Furthermore, this gene may play a role in the survival, proliferation, and/or differentiation of hematopoietic cells in general, and may be of use in the  
10 augmentation of the numbers of stem cells and committed progenitors. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are  
15 related to SEQ ID NO:38 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general  
20 formula of a-b, where a is any integer between 1 to 975 of SEQ ID NO:38, b is an integer of 15 to 989, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:38, and where b is greater than or equal to a + 14.

## 25 FEATURES OF PROTEIN ENCODED BY GENE NO: 29

Preferred polypeptides of the invention comprise the following amino acid sequence: EKVGLLPPTTIAIIQISKDSVSAISDSCLRPSEGRGFRLLKQR (SEQ ID NO: 231). Polynucleotides encoding these polypeptides are also encompassed by the invention.

5           The translation product of this gene shares sequence homology with the superfamily of protocadherin proteins (e.g., See Genbank Acc. No. gil1161230 and gil2995719 (AF052685), and Geneseq Acc. No. R86865). Protocadherins are glycosylated integral membrane proteins, related to Cadherin, which are involved in cell-cell adhesion.

10           When tested against both Jurkat T-cells and U937 Myeloid cell lines, supernatants removed from cells containing this gene activated the GAS assay. Thus, it is likely that this gene activates T-cells and myeloid cells through the Jak-STAT signal transduction pathway. The gamma activating sequence (GAS) is a promoter element found upstream of many genes which are involved in the Jak-STAT pathway.

15           The Jak-STAT pathway is a large, signal transduction pathway involved in the differentiation and proliferation of cells. Therefore, activation of the Jak-STAT pathway, reflected by the binding of the GAS element, can be used to indicate proteins involved in the proliferation and differentiation of cells.

          This gene is expressed primarily in ovarian tumor.

20           Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, reproductive disorders, including ovarian cancer. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing  
25           immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the reproductive



system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., reproductive, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder. Preferred polypeptides of the present invention comprise immunogenic epitopes shown in SEQ ID NO: 115 as residues: Asp-216 to Gly-224, Asp-268 to Asn-274, Thr-285 to Lys-290, Asp-339 to Pro-345, Ile-356 to Pro-361, Arg-371 to Asn-378, Ala-408 to Tyr-417, Pro-429 to Gln-434, Arg-461 to Pro-466, Ala-475 to Ala-482. Polynucleotides encoding said polypeptides are also provided.

The tissue distribution in ovarian tumor tissue, and the homology to a family of protocadherin proteins, indicates that this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis, detection and/or treatment of cancer and other proliferative disorders. The tissue distribution in ovarian tumors additionally indicates that polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis, detection and/or intervention of these tumors, in addition to other tumors where expression has been indicated.

Alternatively, the GAS biological activity demonstrated with Jurkat T-cells and U937 myeloid cells is a strong indicator that the translation product of this gene is involved in the activation of immune system cells, such as T-cells and myeloid cells. Thus, the translation product of this gene may be involved in the regulation of the proliferation; survival; differentiation; and/or activation of potentially all hematopoietic cell lineages, including blood stem cells. Protein, as well as, antibodies directed against the protein may show utility as a tissue-specific marker and/or immunotherapy target for the above listed tissues. In addition, expression of this gene

product in the ovaries may implicate this gene product in normal ovarian function (e.g., endocrine function, egg maturation). Similarly, this gene product may be useful in the treatment of female infertility, and/or could be used as a female contraceptive.

Many polynucleotide sequences, such as EST sequences, are publicly  
 5 available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:39 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or  
 10 more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2034 of SEQ ID NO:39, b is an integer of 15 to 2048, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:39, and where b is greater than or equal to a + 14.

15

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 30**

The translation product of this gene shares sequence homology with bovine brevican protein, which is a proteoglycan of the aggrecan/versican family (See  
 20 Genbank Accession No: gi452821).

Preferred polypeptides of the invention comprise the following amino acid sequence:

RGSEETGSSEGAPSLPATRAPEGTRELEAPSEDNSGRTAPAGTSVQAQPVL  
 PTDSASRGGVAVVPASGDCVPSPCHNGGTCLEEEGVRLCLPGYGGDLCDV  
 25 GLRFCNPGWDAFQGACYKHFSTRRSWEEAETQCRMYGAHLASISTPEEQDFI  
 NNRYREYQWIGLNDRTIEGDFLWSDGVPLLYENWNPQPDSYFLSGENCVV

TR A (SEQ ID NO: 232),

RGESEETGSSEGAPSLPATRAPEGTRELEAPSEDNSGRTAP (SEQ ID NO:  
233), AGTSVQAQPVLPTDSASRGGVAVVPASGDCVPSPCHNGGT (SEQ ID  
NO: 234), CLEEEGVRLCLPGYGGDLCDVGLRFCNPGWDAFQGACYKHF  
(SEQ ID NO: 235),

STRRSWEEAETQCRMYGAHLASISTPEEQDFINNRYREYQWIG (SEQ ID NO:  
236), and/or LNDRTIEGDFLWSDGVPLLYENWNPGQPDSYFLSGENCVVTRA  
(SEQ ID NO: 237). Polynucleotides encoding these polypeptides are also provided.

This gene is expressed primarily in brain and neural tissues such as human  
brain tissue (adult and fetal), frontal cortex tissue, and glioblastoma tissue and to a  
lesser extent in several immune system tissues.

Therefore, polynucleotides and polypeptides of the invention are useful as  
reagents for differential identification of the tissue(s) or cell type(s) present in a  
biological sample and for diagnosis of diseases and conditions which include, but are  
not limited to, brain and neural disorders. Similarly, polypeptides and antibodies  
directed to these polypeptides are useful in providing immunological probes for  
differential identification of the tissue(s) or cell type(s). For a number of disorders of  
the above tissues or cells, particularly of the neural system, expression of this gene at  
significantly higher or lower levels may be routinely detected in certain tissues or cell  
types (e.g., brain, neural, cancerous and wounded tissues) or bodily fluids (e.g.,  
lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell  
sample taken from an individual having such a disorder, relative to the standard gene  
expression level, i.e., the expression level in healthy tissue or bodily fluid from an  
individual not having the disorder.

The tissue distribution in brain and neural tissue, and the homology to bovine  
brevican protein, indicates that polynucleotides and polypeptides corresponding to

this gene are useful for the detection and/or treatment of neural and brain associated disorders. Furthermore, the tissue distribution in neural tissues indicates that polynucleotides and polypeptides corresponding to this gene are useful for the detection/treatment of neurodegenerative disease states and behavioral disorders such as Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette Syndrome, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, the gene or gene product may also play a role in the treatment and/or detection of developmental disorders associated with the developing embryo, or sexually-linked disorders. Elevated expression of this gene product within the frontal cortex of the brain indicates that it may be involved in neuronal survival; synapse formation; conductance; neural differentiation, etc. Such involvement may impact many processes, such as learning and cognition. It may also be useful in the treatment of such neurodegenerative disorders as schizophrenia; ALS; or Alzheimer's. Additionally, antibodies raised against this protein may be useful in the detection of gliosis. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:40 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 620 of SEQ ID NO:40, b is an

integer of 15 to 634, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:40, and where b is greater than or equal to a + 14.

## 5 FEATURES OF PROTEIN ENCODED BY GENE NO: 31

This gene is expressed primarily in ovarian cancer tissue.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, cancer and disorders of the reproductive organs. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the female reproductive system or endocrine system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., reproductive, endocrine, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in ovarian cancer tissue indicates that polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis, detection and/or treatment of disorders of the reproductive organs, as well as cancers thereof. Similarly, polynucleotides and polypeptides corresponding to this gene are useful for the detection, treatment, and/or prevention of various endocrine disorders and

cancers, particularly Addison's disease, Cushing's Syndrome, and disorders and/or cancers of the pancreas (e.g., diabetes mellitus), adrenal cortex, ovaries, pituitary (e.g., hyper-, hypopituitarism), thyroid (e.g., hyper-, hypothyroidism), parathyroid (e.g., hyper-, hypoparathyroidism), hypothalamus, and testes. Additionally,

5 polynucleotides and polypeptides corresponding to this gene are useful for the treatment and diagnosis of conditions concerning proper ovarian function (e.g., endocrine function, egg maturation), as well as cancer. Therefore, this gene product is useful in the treatment of female infertility. This gene product is also useful in assays designed to identify binding agents, as such agents (antagonists) are useful as female  
10 contraceptive agents. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are  
15 related to SEQ ID NO:41 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general  
20 formula of a-b, where a is any integer between 1 to 2749 of SEQ ID NO:41, b is an integer of 15 to 2763, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:41, and where b is greater than or equal to a + 14.

## 25 FEATURES OF PROTEIN ENCODED BY GENE NO: 32

This gene is expressed primarily in neutrophils (IL-1 and LPS induced), endometrial tumor, and pituitary and to a lesser extent in lung cancer, KMH2, NCI CGAP Kid3 (kidney), pregnant uterus, and Hodgkin's lymphoma.

Therefore, polynucleotides and polypeptides of the invention are useful as  
5 reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, cancer and other proliferative disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of  
10 disorders of the above tissues or cells, particularly of the immune system, cancer and other proliferative disorders, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a  
15 disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder. Preferred polypeptides of the present invention comprise immunogenic epitopes shown in SEQ ID NO: 118 as residues: Gly-27 to Phe-35, Asp-64 to Ala-74, Pro-90 to Gly-96, Pro-104 to Pro-120. Polynucleotides encoding said polypeptides are also provided.

20 The tissue distribution indicates that this protein product of this gene is useful for diagnosis and treatment of cancer and other proliferative disorders.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:42 and may have been publicly available prior to conception of  
25 the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is

cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 641 of SEQ ID NO:42, b is an integer of 15 to 655, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:42, and where b is greater than or equal to a + 14.

### FEATURES OF PROTEIN ENCODED BY GENE NO: 33

The translation product of this gene was shown to have homology to a human putative tumor suppressor protein (See Genbank Accession Nos. gil3126876, gil2997698, and sp|O60539|O60539. These Accession Nos., in addition to any references cited therein, are hereby incorporated by reference herein) which is thought to be involved in the regulation of cellular division and proliferation. Preferred polypeptides of the invention comprise the following amino acid sequence:

GTRSSHVPISDSKSIQKSELLGLLKTYNCYHEGKSFQLRHREEEGTLIEGLLNI  
AWGLRRPIRLQMDD REQVHLPSTSW (SEQ ID NO: 238),  
VPISDSKSIQKSELLGLLKTYNCYH (SEQ ID NO: 239), and/or  
FQLRHREEEGTLIEGLLNIAWGLRRPI (SEQ ID NO: 240),

GTRSSHVPISDSKSIQKSELLGLLKTYNCYHEGKSFQLRHREEEGTLIEGLLNI  
AWGLRRPIRLQMDDREQVHLPSTSWMPRRPSCPLGCWSLLLGLSSLSPAA  
ISALQLSVFRKEPSPQNGNITAQGPSIQPVHKAESSTDSSGPLEEAEAPQLMR  
TKSDASCMSQRRPKCRAPGEAQRIRRHRSINGHFYNHKTSVFTPAYGSVTN  
VRVNSTMTTLQVLTLLLNKFRVEDGPSEFALYIVHESGERTKLKDCEYPLISRI  
LHGPCCKIARIFLMEADLGVEVPHEVAQYIKFEMPVLDSFVEKLKEEEEREIHK



LTMKFQALRLTMLQRLEQLVEAK (SEQ ID NO: 241). Polynucleotides encoding these polypeptides are also encompassed by the invention.

The gene encoding the disclosed cDNA is believed to reside on chromosome 10. Accordingly, polynucleotides related to this invention are useful as a marker in  
5 linkage analysis for chromosome 10.

This gene is expressed primarily in stomach, pharynx carcinoma, infant brain, parathyroid tumor, fetal liver spleen, and dendritic cells and to a lesser extent ubiquitously in other tissues.

Therefore, polynucleotides and polypeptides of the invention are useful as  
10 reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, diseases of the stomach, pharynx carcinoma, infant brain, parathyroid tumor, fetal liver spleen, and dendritic cells. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for  
15 differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the digestive, endocrine, hematopoiesis, and immune systems expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., stomach, pharynx, brain, developmental, endocrine, immune, hematopoietic, hepatic, and cancerous and  
20 wounded tissues) or bodily fluids (e.g., serum, amniotic fluid, chyme, bile, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder. Preferred polypeptides of the present invention comprise immunogenic  
25 epitopes shown in SEQ ID NO: 119 as residues: Met-1 to Ser-6, Arg-36 to Gly-44, His-57 to Pro-68, Glu-70 to Ala-75, Arg-80 to Ala-85, Met-88 to Cys-95, Gln-102 to

Ile-111, Ser-169 to Glu-179, Lys-229 to Glu-237. Polynucleotides encoding said polypeptides are also provided.

The tissue distribution in stomach, pharynx carcinoma, infant brain, parathyroid tumor, fetal liver spleen, and dendritic cells indicates that polynucleotides and polypeptides corresponding to this gene are useful for diagnosis and treatment of stomach, such as gastritis, peptic ulcer, neoplasms, or hemapoietic disorders such as anemia and leukemia, or diseases related to dendritic cells, such as for immunity related diseases, particularly those involved in phagocytic defense against microorganisms, antigen pinocytosis, processing, and the presentation to B- and T- lymphocytes, regulation of production of interleukin or cytokines, modulation of inflammatory response, killing of tumor cells, regulation of hematopoiesis and lymphopoiesis, etc. Moreover, the expression within fetal tissue and other cellular sources marked by proliferating cells (i.e., pharynx carcinoma, parathyroid tumor, etc.) indicates this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis, treatment, and/or prevention of developmental diseases and disorders, cancer, and other proliferative conditions. Similarly, developmental tissues rely on decisions involving cell differentiation and/or apoptosis in pattern formation. Dysregulation of apoptosis can result in inappropriate suppression of cell death, as occurs in the development of some cancers, or in failure to control the extent of cell death, as is believed to occur in acquired immunodeficiency and certain neurodegenerative disorders, such as spinal muscular atrophy (SMA). Because of potential roles in proliferation and differentiation, this gene product may have applications in the adult for tissue regeneration and the treatment of cancers. It may also act as a morphogen to control cell and tissue type specification. Therefore, the polynucleotides and polypeptides of the present invention are useful in treating, detecting, and/or preventing said disorders and

conditions, in addition to other types of degenerative conditions. Thus this protein may modulate apoptosis or tissue differentiation and is useful in the detection, treatment, and/or prevention of degenerative or proliferative conditions and diseases.

The protein is useful in modulating the immune response to aberrant polypeptides, as

5 may exist in proliferating and cancerous cells and tissues. The protein can also be used to gain new insight into the regulation of cellular growth and proliferation.

Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly  
10 available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:43 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or  
15 more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2396 of SEQ ID NO:43, b is an integer of 15 to 2410, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:43, and where b is greater than or equal to a + 14.

20

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 34**

The gene encoding the disclosed cDNA is believed to reside on chromosome 4. Accordingly, polynucleotides related to this invention are useful as a marker in

25 linkage analysis for chromosome 4.

This gene is expressed primarily in placenta, uterus and ovary, and to a lesser extent in a variety of other tissues and cell types.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, developmental anomalies or fetal deficiencies, endometrial cancers, reproductive dysfunction, vascular disorders, and pre-natal disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the reproductive system and developing fetus, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., developmental, reproductive, vascular, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, amniotic fluid, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder. Preferred polypeptides of the present invention comprise immunogenic epitopes shown in SEQ ID NO: 120 as residues: Pro-54 to His-67, Pro-73 to Ala-93. Polynucleotides encoding said polypeptides are also provided.

The tissue distribution in placenta, uterus and ovary indicates that polynucleotides and polypeptides corresponding to this gene are useful for the treatment and diagnosis of developmental anomalies or fetal deficiencies, endometrial cancers, reproductive dysfunction and pre-natal disorders. Alternatively, the protein is useful in the detection, treatment, and/or prevention of vascular conditions, which include, but are not limited to, microvascular disease, vascular leak syndrome,

aneurysm, stroke, atherosclerosis, arteriosclerosis, or embolism. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly  
5 available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:44 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or  
10 more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2620 of SEQ ID NO:44, b is an integer of 15 to 2634, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:44, and where b is greater than or equal to a + 14.

15

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 35**

The gene encoding the disclosed cDNA is believed to reside on chromosome  
3. Accordingly, polynucleotides related to this invention are useful as a marker in  
20 linkage analysis for chromosome 3.

This gene is expressed primarily in pregnant uterus and placental tissues.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are  
25 not limited to, pregnancy and developmental disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological

probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the female reproductive system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., reproductive, cancerous and wounded  
5 tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The predominant expression in the pregnant uterus and placental tissues  
10 indicates a role in the treatment and/or detection of pregnancy disorders and developmental disorders. Furthermore, the tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and/or treatment of disorders of the placenta. Specific expression within the placenta indicates that this gene product may play a role in the proper establishment  
15 and maintenance of placental function. Alternately, this gene product may be produced by the placenta and then transported to the embryo, where it may play a crucial role in the development and/or survival of the developing embryo or fetus. Expression of this gene product in a vascular-rich tissue such as the placenta also indicates that this gene product may be produced more generally in endothelial cells  
20 or within the circulation. In such instances, it may play more generalized roles in vascular function, such as in angiogenesis. It may also be produced in the vasculature and have effects on other cells within the circulation, such as hematopoietic cells. It may serve to promote the proliferation, survival, activation, and/or differentiation of hematopoietic cells, as well as other cells throughout the body. Protein, as well as,  
25 antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:45 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 434 of SEQ ID NO:45, b is an integer of 15 to 448, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:45, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 36**

The polynucleotide sequence may have a frame shift. Therefore, preferred polypeptides of the invention comprise the following amino acid sequence:

HASGFLRFCKQATLQFCVVKPLMAVSTVVLQAFGKYRDGDFDVTSGYLYVT  
 IIYNISVSLALYALFLFYF ATRELLSPYSPVLKFF (SEQ ID NO: 242),  
 TRTTSCRTPSTTSHLPTSSTRSSPPWSLGPPGVVAPTASPAPTASVAPATTRRLS  
 CSALMMNSRCGLQWRKCWRHSHGQAVPHLQPHHQARRQLAQCSRRLYLDD  
 QKHSHVASRGTGDSQARPWAFRNIYTWPSLHCPGEGRGHWEGGLCPCCPSC  
 AGGMLGPAAPRPQCLCVDQRLQPSSPSSPRDSQAEVGKPWLPHTPCNTLSDL  
 GSSRLHPPFVHLCPVLDSHPGQEWGCGRSVVLPS (SEQ ID NO: 243),  
 TRTTSCRTPSTTSHLPTSSTRSSPPWSLGPPGVVA (SEQ ID NO: 245),  
 PTASPAPTASVAPATTRRLSCSALMMNSRCGLQWRK (SEQ ID NO: 246),  
 CWRHSHGQAVPHLQPHHQARRQLAQCSRRLYLDDQK (SEQ ID NO: 247),

HSHVASRGTGDSQARPWAFRNIYTWPSLHCPGEGR (SEQ ID NO: 248),

GHWEQGLCPCCPSCAGGMLGPAAPRPQCLCVDQRLQ (SEQ ID NO: 249),

PSSPSSPRDSQAEVGKPWLPHTPCNTLSDLGSSRL (SEQ ID NO: 250),

HPFPVHLCPVLDSHPGQEWGCGRSVVLPS (SEQ ID NO: 251),

5 ILGAGCSGGSAGAIATVRLCPTSSLTTRPGGSWWSAHA AFIYWTRNTHMSLPE  
 ERGTARLAHGPGSIFIHGPACTARARAEDTGSKAYAPAARPVLGACWDQPHP  
 GPNACVWTSGCSLLAPPPRETLRLRSASRGSPTHRAIPCLTWALPACIPSLSTF  
 VQC (SEQ ID NO: 244),

ILGAGCSGGSAGAIATVRLCPTSSLTTRPGGSWWSAHA (SEQ ID NO: 252),

10 AFIYWTRNTHMSLPEERGTARLAHGPGSIFIHGPAC (SEQ ID NO: 253), and/or  
 TARARAEDTGSKAYAPAARPVLGACWDQPHPGPN (SEQ ID NO: 254).

Polynucleotides encoding these polypeptides are also encompassed by the invention.

The gene encoding the disclosed cDNA is believed to reside on chromosome  
 22. Accordingly, polynucleotides related to this invention are useful as a marker in

15 linkage analysis for chromosome 22.

This gene is expressed primarily in fetal and infant brain tissues.

Therefore, polynucleotides and polypeptides of the invention are useful as  
 reagents for differential identification of the tissue(s) or cell type(s) present in a  
 biological sample and for diagnosis and treatment of neurological and developmental  
 20 disorders. Similarly, polypeptides and antibodies directed to these polypeptides are  
 useful in providing immunological probes for differential identification of the  
 tissue(s) or cell type(s). For a number of disorders of the above tissues or cells,  
 particularly of the reproductive system, or CNS, expression of this gene at  
 significantly higher or lower levels may be routinely detected in certain tissues or cell  
 25 types (e.g., reproductive, neural, cancerous and wounded tissues) or bodily fluids  
 (e.g., lymph, amniotic fluid, serum, plasma, urine, synovial fluid and spinal fluid) or



another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder. Preferred polypeptides of the present invention comprise immunogenic epitopes shown in SEQ ID NO: 122 as  
5 residues: Lys-72 to Cys-80, Leu-90 to Pro-96, Ala-110 to Thr-119, Glu-121 to Gly-128, Ser-140 to Lys-147. Polynucleotides encoding said polypeptides are also provided.

The tissue distribution in fetal and infant brain indicates that polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and/or  
10 treatment of neural development disorders. Similarly, the protein product of this gene may be useful for the detection and/or treatment of neurodegenerative disease states and behavioral disorders such as Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette Syndrome, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, panic disorder, learning disabilities, ALS, psychoses,  
15 autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, the gene or gene product may also play a role in the treatment and/or detection of developmental disorders associated with the developing embryo, or sexually-linked disorders. Alternatively, expression within embryonic tissue and other cellular sources marked by proliferating cells indicates that this  
20 protein may play a role in the regulation of cellular division. Similarly, embryonic development also involves decisions involving cell differentiation and/or apoptosis in pattern formation. Thus this protein may also be involved in apoptosis or tissue differentiation and could again be useful in cancer therapy. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or  
25 immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:46 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically  
5 excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2907 of SEQ ID NO:46, b is an integer of 15 to 2921, where both a and b correspond to the positions of nucleotide  
10 residues shown in SEQ ID NO:46, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 37**

15 The gene encoding the disclosed cDNA is believed to reside on chromosome 20. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 20.

This gene is expressed primarily in spleen.

Therefore, polynucleotides and polypeptides of the invention are useful as  
20 reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, systemic infections, systemic immunological-inflammatory disorders, splenomegaly, hematopoietic or lymphopoietic diseases and/or disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing  
25 immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune and

hematopoietic systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, hematopoietic, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken  
5 from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in spleen tissue indicates that polynucleotides and polypeptides corresponding to this gene are useful for diagnosis and treatment of  
10 spleen related diseases or disorders, such as systemic infections, systemic immunological-inflammatory disorders, splenomegaly, hematopoietic or lymphopoietic disorders. Moreover, the expression of this gene product indicates a role in regulating the proliferation; survival; differentiation; and/or activation of hematopoietic cell lineages, including blood stem cells. This gene product may be  
15 involved in the regulation of cytokine production, antigen presentation, or other processes suggesting a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the natural gene product may be involved in immune functions. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immunodeficiency  
20 diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue  
25 injury, demyelination, systemic lupus erythematosus, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, scleroderma and tissues. Moreover, the protein

may represent a secreted factor that influences the differentiation or behavior of other blood cells, or that recruits hematopoietic cells to sites of injury. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:47 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 405 of SEQ ID NO:47, b is an integer of 15 to 419, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:47, and where b is greater than or equal to a + 14.

## **20 FEATURES OF PROTEIN ENCODED BY GENE NO: 38**

Preferred polypeptides of the invention comprise the following amino acid sequence: PHRPPTPQSNFSSHPSSQALTILKRLVGTLLSATGKLVRARXRAWG (SEQ ID NO: 256),

**25** GVMRLRTRQKSRRQRKEKMSRRKSKRKMKRKRRRRQRARGQSQPMRLSFH  
PFPTLVFFQVLTQSWVLSSRRQLLVVRAGPHPPWPLFDLPHSVTPQASHTSV

(SEQ ID NO: 257),

MKRKRRRRQRARGQSQPMRLSFHPFPTLVFFQVLTQSWVLSSR (SEQ ID NO: 258) and/or RQLLVVRAGPHPPWPLFDLPHSVTPQASHTSV (SEQ ID NO: 259).

Polynucleotides encoding these polypeptides are also encompassed by the invention.

5           This gene is expressed primarily in neutrophils.

          Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of hematopoietic or immune disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing

10   immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune and hematopoietic systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., hematopoietic, immune, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum,

15   plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder. Preferred polypeptides of the present invention comprise immunogenic epitopes shown in SEQ ID NO: 124 as residues: Ala-20 to Ala-28.

20   Polynucleotides encoding said polypeptides are also provided.

          The tissue distribution in neutrophils indicates that polynucleotides and polypeptides corresponding to this gene are useful for treatment and diagnosis of disorders of the haemopoietic and immune system. Additionally, the expression in hematopoietic cells and tissues indicates that this protein may play a role in the

25   proliferation, differentiation, and/or survival of hematopoietic cell lineages. In such an event, this gene may be useful in the treatment of lymphoproliferative disorders,

and in the maintenance and differentiation of various hematopoietic lineages from early hematopoietic stem and committed progenitor cells. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

5           Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:48 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is  
10           cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 917 of SEQ ID NO:48, b is an integer of 15 to 931, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:48, and where b is greater than or equal to a + 14.

15

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 39**

Preferred polypeptides of the invention comprise the following amino acid  
20           sequence:  
HHCPALQPGTHTHTHTHTHTHTTRRGMCLVQIYIKLTHRQIPCLCLLGPDSAV  
(SEQ ID NO: 260). Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in Soares ovary tumor NbHOT

25           Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a

biological sample and for diagnosis of ovarian cancer or other diseases or disorders of the female reproductive system. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above  
5 tissues or cells, particularly of the female reproductive system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., ovarian, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene  
10 expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in Soares ovarian tumor indicates that polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and intervention of ovarian tumors, in addition to other tumors where expression has been  
15 indicated. Protein, as well as, antibodies directed against the protein may show utility as a tissue-specific marker and/or immunotherapy target for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:49 and may have been publicly available prior to conception of  
20 the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 746 of SEQ ID NO:49, b is an  
25 integer of 15 to 760, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:49, and where b is greater than or equal to a + 14.

**FEATURES OF PROTEIN ENCODED BY GENE NO: 40**

5           This gene is expressed primarily in fetal retina, lung, brain and T cells.

          Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, neurological, neuroimmune and allergic conditions. Similarly,  
10   polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the CNS, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., CNS, cancerous and wounded tissues) or  
15   bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

          The distribution in lung, brain, fetal retinal, tissues, and T-cells indicates that  
20   polynucleotides and polypeptides corresponding to this gene are useful for the study and treatment of eye, neurodegenerative, neuroimmune, respiratory and immune disorders. Polynucleotides and polypeptides corresponding to this gene are useful for the detection/treatment of neurodegenerative disease states and behavioral disorders such as Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette  
25   Syndrome, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors,



including disorders in feeding, sleep patterns, balance, and perception. In addition, the gene or gene product may also play a role in the treatment and/or detection of developmental disorders associated with the developing embryo, or sexually-linked disorders. Additionally, expression of this gene product in T cells strongly indicates a  
5      role for this protein in immune function and immune surveillance. The tissue distribution in retina also indicates that polynucleotides and polypeptides corresponding to this gene are useful for the treatment and/or detection of eye disorders including blindness, color blindness, impaired vision, short and long sightedness, retinitis pigmentosa, retinitis proliferans, and retinoblastoma,  
10     retinochoroiditis, retinopathy and retinoschisis. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:50 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2465 of SEQ ID NO:50, b is an integer of 15 to 2479, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:50, and where b is greater than or equal to a + 14.

## 25 FEATURES OF PROTEIN ENCODED BY GENE NO: 41

This gene is expressed primarily in human tonsil.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of immune disorders and diseases. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, cancerous and wounded tissues) or bodily fluids (e.g., lymph, saliva, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in tonsils indicates that polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and treatment of a variety of immune system disorders, including immune disorders involving tonsil function. Expression of this gene product in tonsils indicates a role in the regulation of the proliferation; survival; differentiation; and/or activation of potentially all hematopoietic cell lineages, including blood stem cells. This gene product may be involved in the regulation of cytokine production, antigen presentation, or other processes that may also suggest a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the gene or protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immune deficiency diseases such as AIDS, leukemia, rheumatoid

arthritis, inflammatory bowel disease, sepsis, acne, and psoriasis. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:51 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1559 of SEQ ID NO:51, b is an integer of 15 to 1573, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:51, and where b is greater than or equal to a + 14.

## FEATURES OF PROTEIN ENCODED BY GENE NO: 42

This gene is expressed primarily in fetal cochlea, fetal liver/spleen, dendritic cells, and other many immune cell types (e.g., monocytes).

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, hematopoietic disorders; impaired immunity; hearing disorders;

leukemia; inflammation. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at

5 significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., hematopoietic, immune, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from

10 an individual not having the disorder.

The tissue distribution in fetal cochlea, fetal liver/spleen, and dendritic cells indicates that polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and treatment of hematopoietic disorders, possibly coordinating the proliferation, survival, differentiation and activation of a variety of blood cell

15 lineages. Similarly, tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and treatment of cancer and other proliferative disorders. Expression within embryonic tissue and other cellular sources marked by proliferating cells indicates that this protein may play a role in the regulation of cellular division. Additionally, the expression in hematopoietic cells and

20 tissues indicates that this protein may play a role in the proliferation, differentiation, and/or survival of hematopoietic cell lineages. In such an event, this gene may be useful in the treatment of lymphoproliferative disorders, and in the maintenance and differentiation of various hematopoietic lineages from early hematopoietic stem and committed progenitor cells. Similarly, embryonic development also involves

25 decisions involving cell differentiation and/or apoptosis in pattern formation. Thus this protein may also be involved in apoptosis or tissue differentiation and could again

be useful in cancer therapy. . More generally, as evidenced by expression in fetal liver/spleen, this gene may play a role in the survival, proliferation, and/or differentiation of hematopoietic cells in general, and may be of use in augmentation of the numbers of stem cells and committed progenitors. Expression of this gene product in primary dendritic cells also indicates that it may play a role in mediating responses to infection and controlling immunological responses, such as those that occur during immune surveillance. Thus, it may play a role, for example, in T cell activation and costimulation. Finally, expression of this gene product in fetal cochlea indicates a role in hearing and auditory processing, or may simply reflect a more general role in nervous system function. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:52 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1663 of SEQ ID NO:52, b is an integer of 15 to 1677, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:52, and where b is greater than or equal to a + 14.

## **25 FEATURES OF PROTEIN ENCODED BY GENE NO: 43**

This gene is expressed primarily in placenta, uterus and ovarian cancer, as well as a variety of lymphoid and leukocytic tissues, including cancerous sources.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, immunodeficiency, infection, lymphoma, auto-immunity, cancer, inflammation, anemia (leukemia) and other hematopoietic disorders, developmental anomalies or fetal deficiencies and pre-natal disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the hematopoietic, reproductive and immune systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., reproductive, hematopoietic, cancerous and wounded tissues) or bodily fluids (e.g., lymph, amniotic fluid, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder. Preferred polypeptides of the present invention comprise immunogenic epitopes shown in SEQ ID NO: 129 as residues: Arg-35 to Arg-43. Polynucleotides encoding said polypeptides are also provided.

The tissue distribution in placenta, uterus and ovarian cancer tissues indicates that polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis, detection and/or treatment of developmental anomalies, fetal deficiencies, reproductive dysfunctions, and ovarian or endometrial cancers. Similarly, this gene is useful for the diagnosis and/or treatment of disorders of the placenta. Specific expression within the placenta indicates that this gene product may play a role in the

proper establishment and maintenance of placental function. Alternately, this gene product may be produced by the placenta and then transported to the embryo, where it may play a crucial role in the development and/or survival of the developing embryo or fetus. Expression of this gene product in a vascular-rich tissue such as the placenta

5 also indicates that this gene product may be produced more generally in endothelial cells or within the circulation. In such instances, it may play more generalized roles in vascular function, such as in angiogenesis. It may also be produced in the vasculature and have effects on other cells within the circulation, such as hematopoietic cells. It may serve to promote the proliferation, survival, activation, and/or differentiation of

10 hematopoietic cells, as well as other cells throughout the body. Additionally, the tissue distribution in hematopoietic cells and cancers indicates that polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and treatment of cancer and other proliferative disorders. Expression within cellular sources marked by proliferating cells indicates that this protein may play a role in the regulation of

15 cellular division. Additionally, the expression in hematopoietic cells and tissues indicates that this protein may play a role in the proliferation, differentiation, and/or survival of hematopoietic cell lineages. In such an event, this gene may be useful in the treatment of lymphoproliferative disorders, and in the maintenance and differentiation of various hematopoietic lineages from early hematopoietic stem and

20 committed progenitor cells. In addition the expression of this gene product in a variety of immunological tissues would suggest a role in the diagnosis and treatment of immune disorders including: leukemias, lymphomas, auto-immunities, immunodeficiencies (e.g., AIDS), immuno-suppressive conditions (transplantation) and hematopoietic disorders. In addition this gene product may be applicable in

25 conditions of general microbial infection, inflammation or cancer. Protein, as well as,

antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:53 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1878 of SEQ ID NO:53, b is an integer of 15 to 1892, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:53, and where b is greater than or equal to a + 14.

## 15 FEATURES OF PROTEIN ENCODED BY GENE NO: 44

This gene is expressed primarily in ovarian cancer, fetal liver, heart and brain, placenta and to a lesser extent in other normal and transformed cell types.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, aberrant growth and development, tumors. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the reproductive and endocrine systems, expression of this gene at significantly higher or lower levels may be



5 routinely detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for detection of ovarian and other tumors and treatment of developmental and growth disorders, esp. reproductive and endocrine organ and skeletal neoplasms.

10 Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:54 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is  
15 cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1632 of SEQ ID NO:54, b is an integer of 15 to 1646, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:54, and where b is greater than or equal to a + 14.

20

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 45**

25 The translation product of this gene shares homology to ATP-dependent RNA helicases (e.g., See Genbank Acc. No. gnl|PID|d1024893 (AB001636) and gil2407195 (AF017153)).

This gene is expressed primarily in brain tissues, and to a lesser extent in several other tissues including hematopoietic cells and cancers.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, hematopoietic and neural disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the hematopoietic and central nervous system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., hematopoietic, CNS, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in brain indicates that polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis, detection and/or treatment of diseases and/or disorders of the CNS and hematopoietic system, and cancers. Similarly, polynucleotides and polypeptides corresponding to this gene are useful for the detection/treatment of neurodegenerative disease states and behavioral disorders such as Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette Syndrome, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, the gene or gene product may also play a role in the treatment and/or detection of developmental disorders associated with the developing embryo, or sexually-linked

disorders. Additionally, the expression in hematopoietic cells and tissues indicates that this protein may play a role in the proliferation, differentiation, and/or survival of hematopoietic cell lineages. In such an event, this gene may be useful in the treatment of lymphoproliferative disorders, and in the maintenance and differentiation of various hematopoietic lineages from early hematopoietic stem and committed progenitor cells. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:55 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1544 of SEQ ID NO:55, b is an integer of 15 to 1558, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:55, and where b is greater than or equal to a + 14.

## **20 FEATURES OF PROTEIN ENCODED BY GENE NO: 46**

Preferred polypeptides of the invention comprise the following amino acid sequence: HEILQPAV (SEQ ID NO: 261). Polynucleotides encoding these polypeptides are also encompassed by the invention.

**25** This gene is expressed primarily in stomach and cord blood.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of blood and metabolic diseases and disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in

5 providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the gastrointestinal and hematopoietic systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., gastrointestinal, hematopoietic, cancerous and wounded tissues) or bodily fluids (e.g.,

10 lymph, bile, gastric juices, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in stomach and cord blood indicates that

15 polynucleotides and polypeptides corresponding to this gene are useful for study and treatment of hematopoietic, immune and gastrointestinal disorders. Additionally, the expression in hematopoietic cells and tissues indicates that this protein may play a role in the proliferation, differentiation, and/or survival of hematopoietic cell lineages. In such an event, this gene may be useful in the treatment of lymphoproliferative

20 disorders, and in the maintenance and differentiation of various hematopoietic lineages from early hematopoietic stem and committed progenitor cells. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly

25 available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:56 and may have been publicly available prior to conception of

the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general  
5 formula of a-b, where a is any integer between 1 to 739 of SEQ ID NO:56, b is an integer of 15 to 753, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:56, and where b is greater than or equal to a + 14.

## 10 FEATURES OF PROTEIN ENCODED BY GENE NO: 47

The gene encoding the disclosed cDNA is believed to reside on chromosome 1. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 1.

15 This gene is expressed primarily in mesenchymal and/or epithelial cells, particularly from such tissues as bone marrow stroma, kidney, placenta, and breast, as well as in regions of the brain.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a  
20 biological sample and for diagnosis of diseases and conditions which include, but are not limited to, fibrosis; acute renal failure; cardiac degeneration; neurodegenerative disorders; breast cancer; inflammation. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of  
25 the above tissues or cells, particularly of the endocrine, reproductive, and nervous system, expression of this gene at significantly higher or lower levels may be

routinely detected in certain tissues or cell types (e.g., mesenchymal and/or epithelial cells cancerous and wounded tissues) or bodily fluids (e.g., lymph, amniotic fluid, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene

- 5 expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in mesenchymal and/or epithelial cells indicates that polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and/or treatment of a variety of disorders. Expression in mesenchymal cells

10 could implicate this protein in a variety of processes, including fibrosis, tissue extravasation & inflammation, arthritis, osteoporosis, and commitment to and differentiation down numerous lineages of the mesenchymal stem cell, including cartilage, bone, and muscle. In addition, expression in regions of the brain may also implicate this gene in a variety of neurodegenerative disorders and learning

- 15 disabilities. Additionally, the tissue distribution in placenta indicates that polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and/or treatment of disorders of the placenta. Specific expression within the placenta indicates that this gene product may play a role in the proper establishment and maintenance of placental function. Alternately, this gene product may be
- 20 produced by the placenta and then transported to the embryo, where it may play a crucial role in the development and/or survival of the developing embryo or fetus. Expression of this gene product in a vascular-rich tissue such as the placenta also indicates that this gene product may be produced more generally in endothelial cells or within the circulation. In such instances, it may play more generalized roles in
- 25 vascular function, such as in angiogenesis. It may also be produced in the vasculature and have effects on other cells within the circulation, such as hematopoietic cells. It

may serve to promote the proliferation, survival, activation, and/or differentiation of hematopoietic cells, as well as other cells throughout the body. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues

5           Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:57 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is  
10           cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1755 of SEQ ID NO:57, b is an integer of 15 to 1769, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:57, and where b is greater than or equal to a + 14.  
15

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 48**

          This gene is expressed primarily in testis and fetal tissues (e.g. fetal heart;  
20       fetal liver/spleen), and to a lesser extent in hematopoietic cells and tissues.

          Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, developmental abnormalities; hematopoietic disorders; aberrant  
25       cellular proliferation; cancer; and reproductive disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological

probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune and reproductive systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, reproductive, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder. Preferred polypeptides of the present invention comprise immunogenic epitopes shown in SEQ ID NO: 134 as residues: Arg-30 to Cys-42. Polynucleotides encoding said polypeptides are also provided.

The tissue distribution in testis tissue indicates that polynucleotides and polypeptides corresponding to this gene are useful for the treatment and/or diagnosis of reproductive disorders, particularly male reproductive disorders or infertility.

Elevated expression of this gene product in the testis indicates a role in normal testis function, sperm maturation, etc. Similarly, expression of this gene product in fetal tissues indicates a role for this protein in cellular proliferation, which may implicate this gene as a diagnostic or causative agent in the development and progression of cancer. Expression of this gene product in hematopoietic sources such as fetal liver/spleen also indicates a potential role for this protein in hematopoietic proliferation, survival, differentiation, and/or activation. This gene product may be involved in the regulation of cytokine production, antigen presentation, or other processes that may also suggest a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the gene or protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.



Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immune deficiency diseases such as AIDS, leukemia, rheumatoid arthritis, inflammatory bowel disease, sepsis, acne, and psoriasis. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:58 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 612 of SEQ ID NO:58, b is an integer of 15 to 626, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:58, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 49**

This gene is expressed primarily in breast and fetal liver and to a lesser extent in placenta, testes and other normal and transformed cell types.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a

biological sample and for diagnosis of diseases and conditions which include, but are not limited to, immune and reproductive conditions. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune and reproductive systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder. Preferred polypeptides of the present invention comprise immunogenic epitopes shown in SEQ ID NO: 135 as residues: Gly-37 to Ser-53. Polynucleotides encoding said polypeptides are also provided.

The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for study and treatment of inflammatory, immune, and reproductive disorders and neoplasms.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:59 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 620 of SEQ ID NO:59, b is an

integer of 15 to 634, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:59, and where b is greater than or equal to a + 14.

## 5 FEATURES OF PROTEIN ENCODED BY GENE NO: 50

When tested against fibroblast cell lines, supernatants removed from cells containing this gene activated the EGR1 assay. Thus, it is likely that this gene activates fibroblast cells through a signal transduction pathway. Early growth  
10 response 1 (EGR1) is a promoter associated with certain genes that induces various tissues and cell types upon activation, leading the cells to undergo differentiation and proliferation.

This gene is expressed primarily in CD34 depleted cord blood.

Therefore, polynucleotides and polypeptides of the invention are useful as  
15 reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, pathologies of the cardiovascular system, as well as reproductive disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the  
20 tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the vascular, reproductive or immune systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., vascular, reproductive, immune, cancerous and wounded tissues) or bodily fluids (e.g., amniotic fluid, lymph, serum, plasma, urine, synovial fluid and  
25 spinal fluid) or another tissue or cell sample taken from an individual having such a

disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in CD34 depleted cord blood, and the biological activity of supernatants from cells expressing this gene, indicates that polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and/or treatment of cancers and other proliferative disorders. Expression within embryonic tissue and activation of the EGR1 promoter indicates that this protein may play a role in the regulation of cellular division. Additionally, the expression in hematopoietic cells and tissues indicates that this protein may play a role in the proliferation, differentiation, and/or survival of hematopoietic cell lineages. In such an event, this gene may be useful in the treatment of lymphoproliferative disorders, and in the maintenance and differentiation of various hematopoietic lineages from early hematopoietic stem and committed progenitor cells. Similarly, embryonic development also involves decisions involving cell differentiation and/or apoptosis in pattern formation. Thus, this protein may also be involved in apoptosis or tissue differentiation and could again be useful in cancer therapy. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:60 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 613 of SEQ ID NO:60, b is an

integer of 15 to 627, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:60, and where b is greater than or equal to a + 14.

## 5 FEATURES OF PROTEIN ENCODED BY GENE NO: 51

Preferred polypeptides of the invention comprise the following amino acid sequence:

NSRVDPRVRDGLMYQKFRNQFLSFSMYQSFVQFLQYYYQSGCLYRLRALGE

10 RHT (SEQ ID NO: 262). Polynucleotides encoding these polypeptides are also encompassed by the invention.

The gene encoding the disclosed cDNA is believed to reside on chromosome 7. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 7.

15 This gene is expressed primarily in testes and breast tissues, and to a lesser extent in a variety of other cell types and tissues.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are  
20 not limited to, sexual, reproductive and endocrine disorders, as well as cancer of the breast and testes. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the reproductive and endocrine systems, expression of  
25 this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., reproductive, cancerous and wounded tissues) or bodily

fluids (e.g., breast milk, lymph, semen, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder. Preferred

5 polypeptides of the present invention comprise immunogenic epitopes shown in SEQ ID NO: 137 as residues: Lys-76 to Asp-87. Polynucleotides encoding said polypeptides are also provided.

The tissue distribution in breast and testes tissues indicates that polynucleotides and polypeptides corresponding to this gene are useful for the

10 diagnosis, detection and/or treatment of reproductive disorders and endocrine disorders. Polynucleotides and polypeptides corresponding to this gene are useful for the treatment and diagnosis of conditions concerning proper testicular function (e.g., endocrine function, sperm maturation), as well as cancer. Therefore, this gene product is useful in the treatment of male infertility and/or impotence. This gene product is

15 also useful in assays designed to identify binding agents, as such agents (antagonists) are useful as male contraceptive agents. The testes are also a site of active gene expression of transcripts that may be expressed, particularly at low levels, in other tissues of the body. Therefore, this gene product may be expressed in other specific tissues or organs where it may play related functional roles in other processes, such as

20 hematopoiesis, inflammation, bone formation, and kidney function, to name a few possible target indications. Additionally, polynucleotides and polypeptides corresponding to this gene are useful for the detection, treatment, and/or prevention of various endocrine disorders and cancers, particularly Addison's disease, Cushing's Syndrome, and disorders and/or cancers of the pancreas (e.g., diabetes mellitus),

25 adrenal cortex, ovaries, pituitary (e.g., hyper-, hypopituitarism), thyroid (e.g., hyper-, hypothyroidism), parathyroid (e.g., hyper-, hypoparathyroidism), hypothalamus, and

testes. It may also prove to be valuable in the diagnosis and treatment of breast and/or testicular cancers. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

5 Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:61 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is  
10 cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 618 of SEQ ID NO:61, b is an integer of 15 to 632, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:61, and where b is greater than or equal to a + 14.

15

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 52**

Preferred polypeptides of the invention comprise the following amino acid  
20 sequence: ILMPFCGLH (SEQ ID NO: 263). Polynucleotides encoding these polypeptides are also encompassed by the invention.

When tested against U937 Myeloid cell lines and Jurkat T-cell lines, supernatants removed from cells containing this gene activated the GAS assay. Thus, it is likely that this gene activates myeloid cells and T-cells through the Jak-STAT  
25 signal transduction pathway. The gamma activating sequence (GAS) is a promoter element found upstream of many genes which are involved in the Jak-STAT pathway.

The Jak-STAT pathway is a large, signal transduction pathway involved in the differentiation and proliferation of cells. Therefore, activation of the Jak-STAT pathway, reflected by the binding of the GAS element, can be used to indicate proteins involved in the proliferation and differentiation of cells.

5           This gene is expressed primarily in ovarian tumor tissue.

          Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, reproductive disorders, e.g., ovarian tumors. Similarly, polypeptides  
10   and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the reproductive or endocrine systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., reproductive, endocrine,  
15   cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder. Preferred polypeptides of the present invention comprise immunogenic  
20   epitopes shown in SEQ ID NO: 138 as residues: Met-35 to Gly-42, Glu-51 to Ala-56. Polynucleotides encoding said polypeptides are also provided.

          The tissue distribution in ovarian tumor tissue, and the GAS biological activity demonstrated in T-cells and myeloid cell lines, indicates that polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and treatment of  
25   cancer and other proliferative disorders. The Jak-STAT pathway is a large, signal transduction pathway involved in the differentiation and proliferation of cells.



Therefore, activation of the Jak-STAT pathway, reflected by the binding of the GAS element, can be used to indicate proteins involved in the proliferation and differentiation of cells.

Expression within cellular sources marked by proliferating cells, e.g., ovarian  
5 tumors, and the biological activity of this gene indicates that this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis and treatment of cancer and other proliferative disorders. Based on the tissue distribution in ovarian cancer tissue, preferred are antibodies which specifically bind a portion of the translation product of this gene. Also provided is a kit for detecting ovarian  
10 cancer. Such a kit comprises in one embodiment an antibody specific for the translation product of this gene bound to a solid support. Also provided is a method of detecting ovarian cancer in an individual which comprises a step of contacting an antibody specific for the translation product of this gene to a bodily fluid from the individual, preferably serum, and ascertaining whether antibody binds to an antigen  
15 found in the bodily fluid. Preferably the antibody is bound to a solid support and the bodily fluid is serum. The above embodiments, as well as other treatments and diagnostic tests (kits and methods), are more particularly described elsewhere herein. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues, especially in  
20 ovarian cancer.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:62 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically  
25 excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or

more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 692 of SEQ ID NO:62, b is an integer of 15 to 706, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:62, and where b is greater than or equal to a + 14.

5

### FEATURES OF PROTEIN ENCODED BY GENE NO: 53

The translation product of this gene shares sequence homology with a fasting-  
10 inducible gene encoding a membrane associated protein with six transmembrane domains (See Genbank Accession AAF01324).

The gene encoding the disclosed cDNA is believed to reside on chromosome 2. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 2.

15 The polynucleotide sequence of this gene may have a frame shift, therefore, preferred polypeptides of the invention comprise the following amino acid sequence:  
LPLVLPPTPPPPWLPSL (SEQ ID NO: 264),  
TTMYALWRTGPTTSPALLTLLSKGVPRPAAPWTMSPSSVALICLLRYGQLLE  
QSRHSWVNNTALITGCTNAAGLLVVGNFQVDHARSLHYVGAGVAFFAGLLF  
20 VCLHCALSYQGATAPLDLAVAYLRSLAVIAFITLVLSGVFFVHESSQLQHGA  
ALCEWVCVIDILIFYGTFSYEFQAVSSDTLVAALQPTPGRACKSSGSSSTSTHL  
NCAPESIAMI (SEQ ID NO: 265),  
TTMYALWRTGPTTSPALLTLLSKGVPRPAAPWTMSPS (SEQ ID NO: 266),  
SVALICLLRYGQLLEQSRHSWVNNTALITGCTNA (SEQ ID NO: 267),  
25 AGLLVVGNFQVDHARSLHYVGAGVAFFAGLLFVCLHC (SEQ ID NO: 268),  
ALSYQGATAPLDLAVAYLRSLAVIAFITLVLSG (SEQ ID NO: 269),

VFFVHESSQLQHGAALCEWVCVIDILIFYGTFSYEFGAVSS (SEQ ID NO: 270),  
 DTLVAALQPTPGRACKSSGSSSTSTHLNCAPESIAMI (SEQ ID NO: 271),  
 SASCATGSSWSRVGTLGLTPRHSSQAAPTLRASWWLATFRWIMPGLCTTLEL  
 A

5 WPSLRGCSLFACTVLSPTKGPPRWTWLWPICEVCWLSSPLSPWSSVESSLMS  
 RVLSCNMGQPCVSGCVS  
 SISSFSMAPSATSLGQSPQTHWWLHCSLPLAGPASPPGAAAPPPTSTVPPRASL  
 (SEQ ID NO: 272), SASCATGSSWSRVGTLGLTPRHSSQAAPTLRASWWLAT  
 (SEQ ID NO: 273), FRWIMPGLCTTLELAWPSLRGCSLFACTVLSPT (SEQ ID  
 10 NO: 274), KGPPRWTWLWPICEVCWLSSPLSPWSSVESSLMSR (SEQ ID NO:  
 275), VLSCNMGQPCVSGCVSSISSFSMAPSATSLGQSPQ (SEQ ID NO: 276),  
 and/or THWWLHCSLPLAGPASPPGAAAPPPTSTVPPRASL (SEQ ID NO: 277).

Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in ovarian tumor and to a lesser extent in  
 15 caudate nucleus, heart, colorectal tumor and wide range of tissues and organs.

Therefore, polynucleotides and polypeptides of the invention are useful as  
 reagents for differential identification of the tissue(s) or cell type(s) present in a  
 biological sample and for diagnosis of diseases and conditions which include, but are  
 not limited to, reproductive or endocrine disorders. Similarly, polypeptides and  
 20 antibodies directed to these polypeptides are useful in providing immunological  
 probes for differential identification of the tissue(s) or cell type(s). For a number of  
 disorders of the above tissues or cells, particularly of the reproductive or endocrine  
 systems, expression of this gene at significantly higher or lower levels may be  
 routinely detected in certain tissues or cell types (e.g., reproductive, endocrine,  
 25 cancerous and wounded tissues) or bodily fluids (e.g., lymph, amniotic fluid, serum,  
 plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken

from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder. Preferred polypeptides of the present invention comprise immunogenic epitopes shown in SEQ ID NO: 139 as residues: Glu-36 to Lys-55.

- 5 Polynucleotides encoding said polypeptides are also provided.

The tissue distribution in ovarian tumor tissue indicates that polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and/or treatment of reproductive and/or endocrine disorders. Similarly, polynucleotides and polypeptides corresponding to this gene are useful for the treatment and/or diagnosis  
10 of conditions concerning proper ovarian function (e.g., endocrine function, egg maturation), as well as cancer (e.g., ovarian tumors, serous adenocarcinoma, dysgerminoma, embryonal carcinoma, choriocarcinoma, teratoma, etc.). Therefore, this gene product is useful in the treatment of female infertility, sexual dysfunction or sex development disorders. Similarly, the protein is believed to be useful in the  
15 treatment and/or diagnosis of ovarian cancer. The ovaries are also a site of active gene expression of transcripts that may be expressed, particularly at low levels as evidenced by the wide tissue distribution, in other tissues of the body. Accordingly, preferred are antibodies which specifically bind a portion of the translation product of this gene. Also provided is a kit for detecting ovarian cancer. Such a kit comprises in  
20 one embodiment an antibody specific for the translation product of this gene bound to a solid support. Also provided is a method of detecting ovarian cancer in an individual which comprises a step of contacting an antibody specific for the translation product of this gene to a bodily fluid from the individual, preferably serum, and ascertaining whether antibody binds to an antigen found in the bodily  
25 fluid. Preferably the antibody is bound to a solid support and the bodily fluid is serum. The above embodiments, as well as other treatments and diagnostic tests (kits

and methods), are more particularly described elsewhere herein. Furthermore, this gene product may be expressed in other specific tissues or organs where it may play related functional roles in other processes, such as hematopoiesis, inflammation, bone formation, and kidney function, to name a few possible target indications. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:63 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1331 of SEQ ID NO:63, b is an integer of 15 to 1345, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:63, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 54**

Preferred polypeptides of the invention comprise the following amino acid sequence: SCHSGQQSETVSEKK (SEQ ID NO: 278). Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in neutrophils.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a

biological sample and for diagnosis of hematopoietic disorders or immune. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune and hematopoietic system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., hematopoietic, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in neutrophils indicates that polynucleotides and polypeptides corresponding to this gene are useful for treatment and diagnosis of disorders of the immune and haemopoietic system. Additionally, the expression in hematopoietic cells and tissues indicates that this protein may play a role in the proliferation, differentiation, and/or survival of hematopoietic cell lineages. In such an event, this gene may be useful in the treatment of lymphoproliferative disorders, and in the maintenance and differentiation of various hematopoietic lineages from early hematopoietic stem and committed progenitor cells. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:64 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is

cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 759 of SEQ ID NO:64, b is an integer of 15 to 773, where both a and b correspond to the positions of nucleotide  
5 residues shown in SEQ ID NO:64, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 55**

10 The translation product of this gene shares sequence homology with DREG-2, a transcript identified in *Drosophila* that exhibits circadian rhythm expression but has no identified function (See Genbank Accession No.: gil1561732).

The gene encoding the disclosed cDNA is believed to reside on chromosome 9. Accordingly, polynucleotides related to this invention are useful as a marker in  
15 linkage analysis for chromosome 9.

This gene is expressed primarily in fetal tissues such as fetal liver/spleen, brain, kidney, and heart, and to a lesser extent in pancreas tumor and brain.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a  
20 biological sample and for diagnosis of diseases and conditions which include, but are not limited to, developmental, neurological disorders and cancer. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the developmental  
25 and nervous systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., developmental, neural,

cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution primarily in fetal tissue, and its distant homology to a factor whose expression is regulated with regards to the circadian rhythm in *Drosophila*, indicates a critical role in development and in circadian rhythm disturbances associated with shift work, jet lag, blindness, insomnia and old age. The gene product could be used in the treatment of developmental and/or metabolic disorders. Expression in brain indicates a role in the detection/treatment of neurodegenerative disease states and behavioral disorders such as Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder and panic disorder. Finally overexpression in pancreas tumor indicates a role in the treatment and/or detection of pancreas disorders including pancreatic tumors. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:65 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1555 of SEQ ID NO:65, b is an



integer of 15 to 1569, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:65, and where b is greater than or equal to a + 14.

## 5 FEATURES OF PROTEIN ENCODED BY GENE NO: 56

The translation product of this gene was shown to have homology to potential vascular endothelial cell-specific receptors which are thought to be important in vascularization. Preferred polypeptides of the invention comprise the following amino acid sequence: SPPISFTLTSGLPNP (SEQ ID NO: 279). Northern analysis has been performed with polyA mRNA blot. The mRNA size was estimated to be ~ 1.2 kb. The expression was significantly higher in highly vascularized tissues, i.e. heart, lung, placenta, skeletal muscle and much lower in brain, liver, kidney, and pancreas. The Northern blot analysis with total RNA from primary cells showed that this gene was highly expressed in vascular endothelial cells (HUVEC) and HUVEC induced with LPS, but not in vascular smooth muscle cells, fibroblast, or neutrophils. Therefore, its expression is specific to endothelial cells. Northern blot with fetal tissues resulted in the detection of a 1.2 kb mRNA expressed at higher levels in fetal lung and kidney, lower in fetal brain and liver. Polynucleotides encoding these polypeptides are also encompassed by the invention.

The gene encoding the disclosed cDNA is believed to reside on chromosome 8. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 8.

This gene is primarily expressed in dendritic cells, SAOS2 cells, and to a less extent in other tissues.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, vascular, immune, and skeletal-related diseases and/or disorders.

- 5 Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., vascular, immune, skeletal, and  
10 cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

- The tissue distribution in dendritic cells indicates that polynucleotides and  
15 polypeptides corresponding to this gene are useful for the treatment and diagnosis for immune related diseases, particularly those involved in phagocytic defense against microorganisms, antigen pinocytosis, processing, and the presentation to B- and T- lymphocytes, regulation of production of interleukin or cytokines, modulation of inflammatory response, killing of tumor cells, regulation of hematopoiesis and  
20 lymphopoiesis, etc..

- Alternatively, the northern blot expression within highly vascularized cells and tissues indicates the protein is useful in the detection, treatment, and/or prevention of vascular conditions, which include, but are not limited to, microvascular disease, vascular leak syndrome, aneurysm, stroke, atherosclerosis, arteriosclerosis, or  
25 embolism. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:66 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2643 of SEQ ID NO:66, b is an integer of 15 to 2657, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:66, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 57**

This gene is expressed primarily in activated neutrophils.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of infectious and inflammatory conditions. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of hematopoietic or immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., hematopoietic, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression

level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in hematopoietic cells indicates that polynucleotides and polypeptides corresponding to this gene are useful for study and treatment of infectious, inflammatory and other immune disorders. Additionally, the expression in hematopoietic cells and tissues indicates that this protein may play a role in the proliferation, differentiation, and/or survival of hematopoietic cell lineages. In such an event, this gene may be useful in the treatment of lymphoproliferative disorders, and in the maintenance and differentiation of various hematopoietic lineages from early hematopoietic stem and committed progenitor cells. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:67 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1341 of SEQ ID NO:67, b is an integer of 15 to 1355, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:67, and where b is greater than or equal to a + 14.

## **25 FEATURES OF PROTEIN ENCODED BY GENE NO: 58**

Preferred polypeptides of the invention comprise the following amino acid sequence:

QFHTGNSYDHDYAKXXYGNLYYRXSWYACRYRSGIPGSTHASEKIFLSKLIV  
CFLSTWLPFVLLQVIHVXLKVQIPAYIE (SEQ ID NO: 280). Polynucleotides

5 encoding these polypeptides are also provided.

This gene is expressed primarily in small intestine and colon tissues.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are  
10 not limited to, inflammatory bowel disorder; colon cancer; hematopoietic disorders; impaired immunity; and digestive disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune and digestive systems,  
15 expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, gastrointestinal, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in  
20 healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in small intestine and colon tissues indicates that polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and/or treatment of disorders involving the small intestine. This may include diseases associated with digestion and food absorption, as well as  
25 hematopoietic disorders involving the Peyer's patches of the small intestine, or other hematopoietic cells and tissues within the body. Similarly, expression of this gene

product in colon tissue indicates again involvement in digestion, processing, and elimination of food; as well as a potential role for this gene as a diagnostic marker or causative agent in the development of colon cancer, and cancer in general. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:68 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 931 of SEQ ID NO:68, b is an integer of 15 to 945, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:68, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 59**

Preferred polypeptides of the invention comprise the following amino acid sequence: IPIRFVNIFHHSAGCLFIFLI (SEQ ID NO: 281). Polynucleotides encoding these polypeptides are also provided.

This gene is expressed primarily in B-cell lymphoma, human striatum, colon cancer, and to a lesser extent in spleen and several regions of the brain.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a

biological sample and for diagnosis of diseases and conditions which include, but are not limited to, lymphoproliferative, gastrointestinal, and neural diseases and/or disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., lymphoproliferative, gastrointestinal, neural, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder. Preferred polypeptides of the present invention comprise immunogenic epitopes shown in SEQ ID NO: 145 as residues: Thr-36 to Asp-41. Polynucleotides encoding said polypeptides are also provided.

The tissue distribution in B-cell lymphoma indicates that polynucleotides and polypeptides corresponding to this gene are useful for diagnosing and treating disorders of the blood particularly B-cell lymphomas. The uses include bone marrow cell ex- vivo culture, bone marrow transplantation, bone marrow reconstitution, radiotherapy or chemotherapy of neoplasia. The gene product may also be involved in lymphopoiesis, therefore, it can be used in immune disorders such as infection, inflammation, allergy, immunodeficiency etc. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues. Alternatively, the expression within colon cancer tissue and other cellular sources

marked by proliferating cells (i.e., B-cell lymphoma, spleen) indicates this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis, treatment, and/or prevention of developmental diseases and disorders, cancer, and other proliferative conditions. Similarly, developmental tissues rely on

5 decisions involving cell differentiation and/or apoptosis in pattern formation. Dysregulation of apoptosis can result in inappropriate suppression of cell death, as occurs in the development of some cancers, or in failure to control the extent of cell death, as is believed to occur in acquired immunodeficiency and certain neurodegenerative disorders, such as spinal muscular atrophy (SMA). Therefore, the

10 polynucleotides and polypeptides of the present invention are useful in treating, detecting, and/or preventing said disorders and conditions, in addition to other types of degenerative conditions. Thus this protein may modulate apoptosis or tissue differentiation and is useful in the detection, treatment, and/or prevention of degenerative or proliferative conditions and diseases. The protein is useful in

15 modulating the immune response to aberrant polypeptides, as may exist in proliferating and cancerous cells and tissues. The protein can also be used to gain new insight into the regulation of cellular growth and proliferation. Moreover, polynucleotides and polypeptides corresponding to this gene are useful for the detection, treatment, and/or prevention of neurodegenerative disease states and

20 behavioral disorders. In addition, elevated expression of this gene product in regions of the brain indicates it plays a role in normal neural function. Potentially, this gene product is involved in synapse formation, neurotransmission, learning, cognition, homeostasis, or neuronal differentiation or survival. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or

25 immunotherapy targets for the above listed tissues.



Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:69 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1785 of SEQ ID NO:69, b is an integer of 15 to 1799, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:69, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 60

Preferred polypeptides of the invention comprise the following amino acid sequence:

YRIPLAADAGLLQFLQEFSQQTISRTHEIKKQVDGLIRETKATDCRLHNVFNDF  
LMLSNTQFIENRVYDEEVEEPVLKAEAEKTEQEKTREQKEVDLIPKVQEAVN  
YGLQVLDSAFEQLDIKAGNSDSEEDDANGRVELILEPKDLYIDRPLPYLIGSKL  
FMEQEDVGLGELSSEEGSVGSDRGSIVDTEEEKEEEESDEDFAHHS DNEQNQ  
HTTQMSDEEEEDDDGCDLFADSEKEEEDIEDIEENTRPKRSRPTSFADELAARIK  
GDAMGRVDEEPTTLPSGEAKPRKTLKEKKERRTPSDDEEDNLFAPPKLTDED  
FSPFGSGGGLFSGGKGLFDDEDEESDLFMEAPQDRQAGASVKESSSSSKPGK  
KIPAGAVSVFLGDTDVFGAASVPSLKEPQKPEQPTPRKSPYGPPTGLFDDDD  
GDDDDDDFFSAPHSKPSKTRKVQSTADIFGDEEGDLFKEKAVASPEATVSQTD  
ENKARAEKKDLFSSQSASNLKGASLLPGKLPTSVSLFDDEDEEDNLFGGTAA

KKQTLQLQAQREEKAKASELSKKKASALLFSSDEEDQWNIPASQTHLASDSRS  
KGEPRDSGTLQSQEAKAVKKTSLFEEDKEDDLFAIAKDSQKKTQRVSLLFED  
DVDSGGSLSFGSPPTSVPPATKKK (SEQ ID NO:282). Polynucleotides encoding  
these polypeptides are also encompassed by the invention.

5           The gene encoding the disclosed cDNA is believed to reside on chromosome  
10. Accordingly, polynucleotides related to this invention are useful as a marker in  
linkage analysis for chromosome 10.

          This gene is expressed primarily in healing groin wound, fetal heart, and to a  
lesser extent in human collusum and osteoclastoma.

10           Therefore, polynucleotides and polypeptides of the invention are useful as  
reagents for differential identification of the tissue(s) or cell type(s) present in a  
biological sample and for diagnosis of diseases and conditions which include, but are  
not limited to, proliferating, differentiating, and developing diseases and/or disorders.  
Similarly, polypeptides and antibodies directed to these polypeptides are useful in  
15       providing immunological probes for differential identification of the tissue(s) or cell  
type(s). For a number of disorders of the above tissues or cells, particularly of  
developing cells and tissues, expression of this gene at significantly higher or lower  
levels may be routinely detected in certain tissues or cell types (e.g., proliferating,  
differentiating, developing, cardiovascular, and cancerous and wounded tissues) or  
20       bodily fluids (e.g., serum, plasma, amniotic fluid, urine, synovial fluid and spinal  
fluid) or another tissue or cell sample taken from an individual having such a  
disorder, relative to the standard gene expression level, i.e., the expression level in  
healthy tissue or bodily fluid from an individual not having the disorder. Preferred  
polypeptides of the present invention comprise immunogenic epitopes shown in SEQ  
25       ID NO: 146 as residues: Met-1 to Ser-7. Polynucleotides encoding said polypeptides  
are also provided.

The tissue distribution in healing groin wound and fetal heart indicates this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis, treatment, and/or prevention of developmental diseases and disorders, cancer, and other proliferative conditions. Similarly, developmental tissues rely on decisions involving cell differentiation and/or apoptosis in pattern formation. Dysregulation of apoptosis can result in inappropriate suppression of cell death, as occurs in the development of some cancers, or in failure to control the extent of cell death, as is believed to occur in acquired immunodeficiency and certain neurodegenerative disorders, such as spinal muscular atrophy (SMA). Because of potential roles in proliferation and differentiation, this gene product may have applications in the adult for tissue regeneration and the treatment of cancers. It may also act as a morphogen to control cell and tissue type specification. Therefore, the polynucleotides and polypeptides of the present invention are useful in treating, detecting, and/or preventing said disorders and conditions, in addition to other types of degenerative conditions. Thus this protein may modulate apoptosis or tissue differentiation and is useful in the detection, treatment, and/or prevention of degenerative or proliferative conditions and diseases. The protein is useful in modulating the immune response to aberrant polypeptides, as may exist in proliferating and cancerous cells and tissues. The protein can also be used to gain new insight into the regulation of cellular growth and proliferation. The protein is useful in the treatment, detection, and/or prevention of cardiovascular diseases/disorders. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:70 and may have been publicly available prior to conception of

the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general  
5 formula of a-b, where a is any integer between 1 to 1970 of SEQ ID NO:70, b is an integer of 15 to 1984, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:70, and where b is greater than or equal to a + 14.

## 10 FEATURES OF PROTEIN ENCODED BY GENE NO: 61

The translation product of this gene shares sequence homology with "protein associated with Myc", or Pam, which is thought to function in the nucleus to control transcriptional activation of MYC.

15 Preferred polypeptides of the invention comprise the following amino acid sequence:

FLPDHPAKPPSSLVHSPFVFGXPLSFQQPQLQKSPSRNLASRERIYKNYGVAGP  
ASALSSLSHKLKGDRGNISTSSKPASTSGKSELSSKHSRSLKPDGRMSRTTAD  
QKKPRGTESLSASESLILKSDAAKLRSDSHSRSLSPNHNTLQTLKSDGRMPSSS

20 RAESPGPGSRLHLLSQRLSQQ (SEQ ID NO: 283),

FLPDHPAKPPSSLVHSPFVFGXPLSFQQPQLQKSPSRNLASRERIYKNYGVAGP  
ASALSS (SEQ ID NO: 284),and/or

LSHKLKGDRGNISTSSKPASTSGKSELSSKHSRSLKPDGRMSRTTADQKKPRG  
TESLSAS (SEQ ID NO: 285). Polynucleotides encoding these polypeptides are also

25 provided.

This gene is expressed primarily in fetal liver/spleen tissue, and to a lesser extent in human amygdala tissue.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, immune and neural disorders, and cancers. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune and neural systems, and cancer, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, neural, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in immune and neural tissues, and the homology to a protein involved in transcriptional activation of MYC, indicates that polynucleotides and polypeptides corresponding to this gene are useful for the detection and/or treatment of disorders of the immune and neural systems, as well as for the detection and/or treatment of cancer. Furthermore, homology with a protein associated with MYC activation indicates that this protein may play a rôle in the regulation of cellular division, and may show utility in the diagnosis and treatment of cancer and other proliferative disorders. Thus, this protein may also be involved in apoptosis or tissue differentiation and could again be useful in cancer therapy. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:71 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2070 of SEQ ID NO:71, b is an integer of 15 to 2084, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:71, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 62**

This gene is expressed primarily in uterus, skeletal, and to a lesser extent in melanocyte and testis.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, reproductive, skeletal, and immune diseases and/or disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the reproductive system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., reproductive, skeletal, immune, and cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma,

seminal fluid, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder. Preferred polypeptides of the present invention  
5 comprise immunogenic epitopes shown in SEQ ID NO: 148 as residues: Asp-40 to Tyr-46. Polynucleotides encoding said polypeptides are also provided.

The tissue distribution in uterus and testis indicates polynucleotides and polypeptides corresponding to this gene are useful in the detection, treatment, and/or prevention of a variety of reproductive and developmental diseases and/or disorders  
10 which include, but are not limited to the following: infertility, hormonal imbalances, premature labor, etc. This gene product is also useful in assays designed to identify binding agents, as such agents (antagonists) are useful as male contraceptive agents. Similarly, the protein is believed to be useful in the treatment and/or diagnosis of testicular cancer. The testes are also a site of active gene expression of transcripts that  
15 may be expressed, particularly at low levels, in other tissues of the body. Therefore, this gene product may be expressed in other specific tissues or organs where it may play related functional roles in other processes, such as hematopoiesis, inflammation, bone formation, and kidney function, to name a few possible target indications. Protein, as well as, antibodies directed against the protein may show utility as a tumor  
20 marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:72 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically  
25 excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or

more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 720 of SEQ ID NO:72, b is an integer of 15 to 734, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:72, and where b is greater than or equal to a + 14.



Table 1

Gene No.	cDNA Clone ID	ATCC Deposit No.:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
1	HDPMA04	PTA-1543 03/21/00	pCMVSPor t 3.0	11	1538	1	1538	332	332	87	1	20	21	262
2	HEMFQ46	PTA-1544 03/21/00	Uni-ZAP XR	12	1047	1	1047	72	72	88	1	24	25	205
3	HSYAV50	PTA-1544 03/21/00	pCMVSPor t 3.0	13	2801	1	2801	155	155	89	1	23	24	672
4	HKAJK47	PTA-1544 03/21/00	pCMVSPor t 2.0	14	1441	1	1441	155	155	90	1	21	22	386
5	HCGMF16	PTA-1544 03/21/00	pCMVSPor t 2.0	15	3227	1	3227	178	178	91	1	17	18	95
6	HMSGU01	PTA-1544 03/21/00	Uni-ZAP XR	16	1654	1	1654	135	135	92	1	23	24	120
7	HNTCE26	PTA-1544 03/21/00	pCMVSPor t 3.0	17	1763	1	1763	57	57	93	1	28	29	121
8	HPTTI70	PTA-1543 03/21/00	Uni-ZAP XR	18	703	1	703	44	44	94	1	37	38	101

Gene No.	cDNA Clone ID	ATCC Deposit No.:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
9	HNSAD53	PTA-1544 03/21/00	pSport1	19	774	11	734	30	30	95	1	20	21	185
10	HTEBV72	PTA-1543 03/21/00	Uni-ZAP XR	20	1549	1	1549	20	20	96	1	19	20	231
11	HCE3Z61	PTA-1544 03/21/00	Uni-ZAP XR	21	1189	1	1189	120	120	97	1	22	23	136
12	HSSGD52	PTA-1543 03/21/00	Uni-ZAP XR	22	2460	105	2460	338	338	98	1	34	35	606
13	HAPSA79	PTA-1543 03/21/00	Uni-ZAP XR	23	4386	1	4386	468	468	99	1	30	31	310
13	HAPSA79	PTA-499 08/11/99	Uni-ZAP XR	73	4385	1	4385	468	468	149	1	30	31	310
13	HAPSA79	PTA-322 07/09/99	Uni-ZAP XR	74	4386	1	4386	468	468	150	1	30	31	310
14	HASAU84	PTA-1543 03/21/00	Uni-ZAP XR	24	2462	1	1293	150	150	100	1	32	33	247
15	HLWEA51	PTA-1544 03/21/00	pCMVSPort 3.0	25	2635	1	2635	258	258	101	1	19	20	559
16	HNFIZ34	PTA-1544 03/21/00	pBluescript	26	2707	1	2707	93	93	102	1	29	30	71
17	HTELS08	PTA-1544 03/21/00	Uni-ZAP XR	27	1898	1	1898	15	15	103	1	17	18	158

Gene No.	cDNA Clone ID	ATCC Deposit No:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
18	HTLFE57	PTA-1543 03/21/00	Uni-ZAP XR	28	2298	1157	2214	189	189	104	1	18	19	170
18	HTLEJ24	203918 04/08/99	Uni-ZAP XR	75	928	1	928	110	110	151	1	18	19	170
19	HTADW91	PTA-1543 03/21/00	Uni-ZAP XR	29	1481	54	1481	89	89	105	1	22	23	354
20	HUFBY15	PTA-1543 03/21/00	pSport1	30	1012	1	1012	74	74	106	1	26	27	145
21	HELHD85	PTA-1544 03/21/00	Uni-ZAP XR	31	1886	1	1886	41	41	107	1	25	26	79
22	HOFNY91	PTA-1544 03/21/00	pCMVSPort 2.0	32	2406	1	2406	64	64	108	1	14	15	82
23	HEGAK44	PTA-1544 03/21/00	Uni-ZAP XR	33	2636	1	2607	73	73	109	1	25	26	114
23	HEGAK44	PTA-163 06/01/99	Uni-ZAP XR	76	2636	1	2607	73	73	152	1	25	26	114
24	HETBA14	PTA-1544 03/21/00	Uni-ZAP XR	34	1461	1	1461	118	118	110	1	35	36	163
25	HBAFV19	PTA-1543 03/21/00	pSport1	35	953	1	953	6	6	111	1	33	34	258
26	HTXDO17	PTA-1543 03/21/00	Uni-ZAP XR	36	1340	1	1340	169	169	112	1	46	47	96

Gene No.	cDNA Clone ID	ATCC Deposit No.:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
26	HTXDO17	203959 04/26/99	Uni-ZAP XR	77	1320	1	1320	149	149	153	1	46	47	56
27	HE8DS15	PTA-1544 03/21/00	Uni-ZAP XR	37	2199	1	2199	91	91	113	1	24	25	72
28	HLDOW79	PTA-1544 03/21/00	pCMVSPor t3.0	38	989	1	989	43	43	114	1	21	22	275
29	HOFND85	PTA-1544 03/21/00	pCMVSPor t2.0	39	2048	1	2048	167	167	115	1	26	27	627
30	HBIBU30	PTA-1543 03/21/00	Uni-ZAP XR	40	634	1	634	202	202	116	1	19	20	51
31	HODFG71	PTA-1544 03/21/00	Uni-ZAP XR	41	2763	1	2763	470	470	117	1	19	20	74
32	HNHGE28	PTA-1544 03/21/00	Uni-ZAP XR	42	655	1	655	294	294	118	1	20	21	120
33	HACBZ59	PTA-1543 03/21/00	Uni-ZAP XR	43	2410	1	2410	244	244	119	1	29	30	263
34	HHFDL91	PTA-1543 03/21/00	Uni-ZAP XR	44	2634	1	2634	185	185	120	1	21	22	95
35	HYASD09	PTA-1543 03/21/00	pSport1	45	448	1	448	243	243	121	1	26	27	60
36	HDPCL63	PTA-1544 03/21/00	pCMVSPor t3.0	46	2921	1	2921	260	260	122	1	17	18	157

Gene No.	cDNA Clone ID	ATCC Deposit No.:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
36	HBIBB20	PTA-794 09/27/99	Uni-ZAP XR	78	1259	1	1259		153	154	1	30	31	71
37	HBDAD07	PTA-1543 03/21/00	pSport1	47	419	1	419	23	23	123	1	18	19	115
38	HNGLM62	PTA-1543 03/21/00	Uni-ZAP XR	48	931	1	931	419	419	124	1	22	23	90
39	HTLIQ05	PTA-1543 03/21/00	Uni-ZAP XR	49	760	1	760	323	323	125	1	23	24	96
40	HTGAM78	PTA-1543 03/21/00	Uni-ZAP XR	50	2479	108	2479	340	340	126	1	28	29	43
41	HTOHG09	PTA-1543 03/21/00	Uni-ZAP XR	51	1573	1	1573	116	116	127	1	20	21	41
42	HWBFX31	PTA-1543 03/21/00	pCMV/Spor t 3.0	52	1677	1	1677	271	271	128	1	19	20	52
43	HLHDP16	PTA-1544 03/21/00	Uni-ZAP XR	53	1892	1	1892	62	62	129	1	17	18	43
44	HSDBC88	PTA-1544 03/21/00	Uni-ZAP XR	54	1646	1	1646	32	32	130	1	27	28	48
44	HSIDL71	203181 09/09/98	Uni-ZAP XR	79	1420	6	1420	105	105	155	1	43	44	155
45	HOVBX78	PTA-1544 03/21/00	pSport1	55	1558	1	1558	203	203	131	1	33	34	410

Gene No.	cDNA Clone ID	ATCC Deposit No:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
45	HOVBX78	PTA-1544 03/21/00	pSport1	80	1183	1	1183	193	193	156	1	40	41	102
46	HRODZ89	PTA-1543 03/21/00	Uni-ZAP XR	56	753	1	753	27	27	132	1	25	26	64
47	HWADJ89	PTA-1543 03/21/00	pCMVSPor t3.0	57	1769	529	1769	581	581	133	1	22	23	43
48	HYABE50	PTA-1543 03/21/00	pCMVSPor t3.0	58	626	1	626	144	144	134	1	17	18	58
48	HYABE50	203959 04/26/99	pCMVSPor t3.0	81	1881	1256	1881	1390	1390	157	1	17	18	58
49	HSJAQ17	PTA-1544 03/21/00	Uni-ZAP XR	59	634	1	634	135	135	135	1	20	21	103
50	HCUGM86	PTA-1544 03/21/00	ZAP Express	60	627	1	627	91	91	136	1	24	25	44
51	HLDQC46	PTA-1544 03/21/00	pCMVSPor t3.0	61	632	1	632	163	163	137	1	34	35	87
52	HOFOA59	PTA-1544 03/21/00	pCMVSPor t2.0	62	706	1	706	163	163	138	1	24	25	65
53	HFABG18	PTA-1544 03/21/00	Uni-ZAP XR	63	1345	1	1345	53	53	139	1	26	27	87
54	HNHLY33	PTA-1543 03/21/00	Uni-ZAP XR	64	773	1	773	323	323	140	1	26	27	56

Gene No.	cDNA Clone ID	ATCC Deposit No:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
55	HFCFJ18	PTA-1543 03/21/00	Uni-ZAP XR	65	1569	318	1569	361	361	141	1	25	26	45
55	HFCFJ18	209651 03/04/98	Uni-ZAP XR	82	1433	170	1433	206	206	158	1	25	26	45
56	HANGG89	PTA-1543 03/21/00	pSport1	66	2657	348	2398	520	520	142	1	43	44	52
56	HEOMP42	PTA-181 06/07/99	pSport1	83	2454	1	2110	125	125	159	1	23	24	98
56	HPRAL78	209195 08/01/97	Uni-ZAP XR	84	1775	1038	1775	70	70	160	1	29	30	392
56	HDTAT90	209746 04/07/98	pCMVSpot 2.0	85	1379	8	1379	78	78	161	1	26	27	434
57	HNHOD46	PTA-1543 03/21/00	Uni-ZAP XR	67	1355	1	1355	12	12	143	1	20	21	80
58	HWLQU40	PTA-1543 03/21/00	pSport1	68	945	1	945	262	262	144	1	19	20	64
58	HAMHE82	PTA-909 11/02/99	pCMVSpot 3.0	86	700	1	700	129	129	162	1	18	19	150
59	HL YBI58	PTA-1543 03/21/00	pSport1	69	1799	1	1799	144	144	145	1	38	39	51
60	HMSGK61	PTA-1543 03/21/00	Uni-ZAP XR	70	1984	1	1984	123	123	146	1	32	33	78

Gene No.	cDNA Clone ID	ATCC Deposit No.:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
61	HJBG14	PTA-1543 03/21/00	pCMVSPort 3.0	71	2084	1	2084	197	197	147	1	22	23	47
62	HE9NN84	PTA-1543 03/21/00	Uni-ZAP XR	72	734	1	734	380	380	148	1	38	39	53



Table 1 summarizes the information corresponding to each "Gene No." described above. The nucleotide sequence identified as "NT SEQ ID NO:X" was assembled from partially homologous ("overlapping") sequences obtained from the "cDNA clone ID" identified in Table 1 and, in some cases, from additional related DNA clones. The overlapping sequences were assembled into a single contiguous sequence of high redundancy (usually three to five overlapping sequences at each nucleotide position), resulting in a final sequence identified as SEQ ID NO:X.

The cDNA Clone ID was deposited on the date and given the corresponding deposit number listed in "ATCC Deposit No:Z and Date." Some of the deposits contain multiple different clones corresponding to the same gene. "Vector" refers to the type of vector contained in the cDNA Clone ID.

"Total NT Seq." refers to the total number of nucleotides in the contig identified by "Gene No." The deposited clone may contain all or most of these sequences, reflected by the nucleotide position indicated as "5' NT of Clone Seq." and the "3' NT of Clone Seq." of SEQ ID NO:X. The nucleotide position of SEQ ID NO:X of the putative start codon (methionine) is identified as "5' NT of Start Codon." Similarly, the nucleotide position of SEQ ID NO:X of the predicted signal sequence is identified as "5' NT of First AA of Signal Pep."

The translated amino acid sequence, beginning with the methionine, is identified as "AA SEQ ID NO:Y," although other reading frames can also be easily translated using known molecular biology techniques. The polypeptides produced by these alternative open reading frames are specifically contemplated by the present invention.

The first and last amino acid position of SEQ ID NO:Y of the predicted signal peptide is identified as "First AA of Sig Pep" and "Last AA of Sig Pep." The predicted first amino acid position of SEQ ID NO:Y of the secreted portion is

identified as "Predicted First AA of Secreted Portion." Finally, the amino acid position of SEQ ID NO:Y of the last amino acid in the open reading frame is identified as "Last AA of ORF."

SEQ ID NO:X (where X may be any of the polynucleotide sequences disclosed in the sequence listing) and the translated SEQ ID NO:Y (where Y may be any of the polypeptide sequences disclosed in the sequence listing) are sufficiently accurate and otherwise suitable for a variety of uses well known in the art and described further below. For instance, SEQ ID NO:X is useful for designing nucleic acid hybridization probes that will detect nucleic acid sequences contained in SEQ ID NO:X or the cDNA contained in the deposited clone. These probes will also hybridize to nucleic acid molecules in biological samples, thereby enabling a variety of forensic and diagnostic methods of the invention. Similarly, polypeptides identified from SEQ ID NO:Y may be used, for example, to generate antibodies which bind specifically to proteins containing the polypeptides and the secreted proteins encoded by the cDNA clones identified in Table 1.

Nevertheless, DNA sequences generated by sequencing reactions can contain sequencing errors. The errors exist as misidentified nucleotides, or as insertions or deletions of nucleotides in the generated DNA sequence. The erroneously inserted or deleted nucleotides cause frame shifts in the reading frames of the predicted amino acid sequence. In these cases, the predicted amino acid sequence diverges from the actual amino acid sequence, even though the generated DNA sequence may be greater than 99.9% identical to the actual DNA sequence (for example, one base insertion or deletion in an open reading frame of over 1000 bases).

Accordingly, for those applications requiring precision in the nucleotide sequence or the amino acid sequence, the present invention provides not only the generated nucleotide sequence identified as SEQ ID NO:X and the predicted

translated amino acid sequence identified as SEQ ID NO:Y, but also a sample of plasmid DNA containing a human cDNA of the invention deposited with the ATCC, as set forth in Table 1. The nucleotide sequence of each deposited clone can readily be determined by sequencing the deposited clone in accordance with known methods.

- 5 The predicted amino acid sequence can then be verified from such deposits. Moreover, the amino acid sequence of the protein encoded by a particular clone can also be directly determined by peptide sequencing or by expressing the protein in a suitable host cell containing the deposited human cDNA, collecting the protein, and determining its sequence.

- 10 The present invention also relates to the genes corresponding to SEQ ID NO:X, SEQ ID NO:Y, or the deposited clone. The corresponding gene can be isolated in accordance with known methods using the sequence information disclosed herein. Such methods include preparing probes or primers from the disclosed sequence and identifying or amplifying the corresponding gene from appropriate  
15 sources of genomic material.

- Also provided in the present invention are allelic variants, orthologs, and/or species homologs. Procedures known in the art can be used to obtain full-length genes, allelic variants, splice variants, full-length coding portions, orthologs, and/or species homologs of genes corresponding to SEQ ID NO:X, SEQ ID NO:Y, or a  
20 deposited clone, using information from the sequences disclosed herein or the clones deposited with the ATCC. For example, allelic variants and/or species homologs may be isolated and identified by making suitable probes or primers from the sequences provided herein and screening a suitable nucleic acid source for allelic variants and/or the desired homologue.

- 25 The polypeptides of the invention can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly

produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides are well understood in the art.

The polypeptides may be in the form of the secreted protein, including the mature form, or may be a part of a larger protein, such as a fusion protein (see below). It is often advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification, such as multiple histidine residues, or an additional sequence for stability during recombinant production.

The polypeptides of the present invention are preferably provided in an isolated form, and preferably are substantially purified. A recombinantly produced version of a polypeptide, including the secreted polypeptide, can be substantially purified using techniques described herein or otherwise known in the art, such as, for example, by the one-step method described in Smith and Johnson, Gene 67:31-40 (1988). Polypeptides of the invention also can be purified from natural, synthetic or recombinant sources using techniques described herein or otherwise known in the art, such as, for example, antibodies of the invention raised against the secreted protein.

The present invention provides a polynucleotide comprising, or alternatively consisting of, the nucleic acid sequence of SEQ ID NO:X, and/or a cDNA contained in ATCC deposit Z. The present invention also provides a polypeptide comprising, or alternatively, consisting of, the polypeptide sequence of SEQ ID NO:Y and/or a polypeptide encoded by the cDNA contained in ATCC deposit Z. Polynucleotides encoding a polypeptide comprising, or alternatively consisting of the polypeptide sequence of SEQ ID NO:Y and/or a polypeptide sequence encoded by the cDNA contained in ATCC deposit Z are also encompassed by the invention.

#### **Signal Sequences**

The present invention also encompasses mature forms of the polypeptide having the polypeptide sequence of SEQ ID NO:Y and/or the polypeptide sequence encoded by the cDNA in a deposited clone. Polynucleotides encoding the mature forms (such as, for example, the polynucleotide sequence in SEQ ID NO:X and/or the polynucleotide sequence contained in the cDNA of a deposited clone) are also encompassed by the invention. According to the signal hypothesis, proteins secreted by mammalian cells have a signal or secretory leader sequence which is cleaved from the mature protein once export of the growing protein chain across the rough endoplasmic reticulum has been initiated. Most mammalian cells and even insect cells cleave secreted proteins with the same specificity. However, in some cases, cleavage of a secreted protein is not entirely uniform, which results in two or more mature species of the protein. Further, it has long been known that cleavage specificity of a secreted protein is ultimately determined by the primary structure of the complete protein, that is, it is inherent in the amino acid sequence of the polypeptide.

Methods for predicting whether a protein has a signal sequence, as well as the cleavage point for that sequence, are available. For instance, the method of McGeoch, *Virus Res.* 3:271-286 (1985), uses the information from a short N-terminal charged region and a subsequent uncharged region of the complete (uncleaved) protein. The method of von Heinje, *Nucleic Acids Res.* 14:4683-4690 (1986) uses the information from the residues surrounding the cleavage site, typically residues -13 to +2, where +1 indicates the amino terminus of the secreted protein. The accuracy of predicting the cleavage points of known mammalian secretory proteins for each of these methods is in the range of 75-80%. (von Heinje, *supra*.) However, the two methods do not always produce the same predicted cleavage point(s) for a given protein.

In the present case, the deduced amino acid sequence of the secreted polypeptide was analyzed by a computer program called SignalP (Henrik Nielsen et al., Protein Engineering 10:1-6 (1997)), which predicts the cellular location of a protein based on the amino acid sequence. As part of this computational prediction of localization, the methods of McGeoch and von Heinje are incorporated. The analysis of the amino acid sequences of the secreted proteins described herein by this program provided the results shown in Table 1.

As one of ordinary skill would appreciate, however, cleavage sites sometimes vary from organism to organism and cannot be predicted with absolute certainty. Accordingly, the present invention provides secreted polypeptides having a sequence shown in SEQ ID NO:Y which have an N-terminus beginning within 5 residues (i.e., + or - 5 residues) of the predicted cleavage point. Similarly, it is also recognized that in some cases, cleavage of the signal sequence from a secreted protein is not entirely uniform, resulting in more than one secreted species. These polypeptides, and the polynucleotides encoding such polypeptides, are contemplated by the present invention.

Moreover, the signal sequence identified by the above analysis may not necessarily predict the naturally occurring signal sequence. For example, the naturally occurring signal sequence may be further upstream from the predicted signal sequence. However, it is likely that the predicted signal sequence will be capable of directing the secreted protein to the ER. Nonetheless, the present invention provides the mature protein produced by expression of the polynucleotide sequence of SEQ ID NO:X and/or the polynucleotide sequence contained in the cDNA of a deposited clone, in a mammalian cell (e.g., COS cells, as described below). These polypeptides, and the polynucleotides encoding such polypeptides, are contemplated by the present invention.

### Polynucleotide and Polypeptide Variants

The present invention is directed to variants of the polynucleotide sequence disclosed in SEQ ID NO:X, the complementary strand thereto, and/or the cDNA  
5 sequence contained in a deposited clone.

The present invention also encompasses variants of the polypeptide sequence disclosed in SEQ ID NO:Y and/or encoded by a deposited clone.

"Variant" refers to a polynucleotide or polypeptide differing from the polynucleotide or polypeptide of the present invention, but retaining essential  
10 properties thereof. Generally, variants are overall closely similar, and, in many regions, identical to the polynucleotide or polypeptide of the present invention.

The present invention is also directed to nucleic acid molecules which comprise, or alternatively consist of, a nucleotide sequence which is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to, for example, the nucleotide  
15 coding sequence in SEQ ID NO:X or the complementary strand thereto, the nucleotide coding sequence contained in a deposited cDNA clone or the complementary strand thereto, a nucleotide sequence encoding the polypeptide of SEQ ID NO:Y, a nucleotide sequence encoding the polypeptide encoded by the cDNA contained in a deposited clone, and/or polynucleotide fragments of any of  
20 these nucleic acid molecules (e.g., those fragments described herein).

Polynucleotides which hybridize to these nucleic acid molecules under stringent hybridization conditions or lower stringency conditions are also encompassed by the invention, as are polypeptides encoded by these polynucleotides.

The present invention is also directed to polypeptides which comprise, or  
25 alternatively consist of, an amino acid sequence which is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, for example, the polypeptide sequence

shown in SEQ ID NO:Y, the polypeptide sequence encoded by the cDNA contained in a deposited clone, and/or polypeptide fragments of any of these polypeptides (e.g., those fragments described herein).

By a nucleic acid having a nucleotide sequence at least, for example, 95%  
5 "identical" to a reference nucleotide sequence of the present invention, it is intended that the nucleotide sequence of the nucleic acid is identical to the reference sequence except that the nucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence encoding the polypeptide. In other words, to obtain a nucleic acid having a nucleotide sequence at least 95%  
10 identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. The query sequence may be an entire sequence shown in Table 1, the ORF (open reading frame), or any fragment specified  
15 as described herein.

As a practical matter, whether any particular nucleic acid molecule or polypeptide is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to a nucleotide sequence of the present invention can be determined conventionally using known computer programs. A preferred method for determining the best  
20 overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag et al. (Comp. App. Biosci. 6:237-245(1990)). In a sequence alignment the query and subject sequences are both DNA sequences. An RNA sequence can be compared by  
25 converting U's to T's. The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB alignment of DNA sequences to



calculate percent identity are: Matrix=Unitary, k-tuple=4, Mismatch Penalty=1, Joining Penalty=30, Randomization Group Length=0, Cutoff Score=1, Gap Penalty=5, Gap Size Penalty 0.05, Window Size=500 or the length of the subject nucleotide sequence, whichever is shorter.

5           If the subject sequence is shorter than the query sequence because of 5' or 3' deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for 5' and 3' truncations of the subject sequence when calculating percent identity. For subject sequences truncated at the 5' or 3' ends, relative to the query sequence, the percent  
10 identity is corrected by calculating the number of bases of the query sequence that are 5' and 3' of the subject sequence, which are not matched/aligned, as a percent of the total bases of the query sequence. Whether a nucleotide is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using  
15 the specified parameters, to arrive at a final percent identity score. This corrected score is what is used for the purposes of the present invention. Only bases outside the 5' and 3' bases of the subject sequence, as displayed by the FASTDB alignment, which are not matched/aligned with the query sequence, are calculated for the purposes of manually adjusting the percent identity score.

20           For example, a 90 base subject sequence is aligned to a 100 base query sequence to determine percent identity. The deletions occur at the 5' end of the subject sequence and therefore, the FASTDB alignment does not show a matched/alignment of the first 10 bases at 5' end. The 10 unpaired bases represent 10% of the sequence (number of bases at the 5' and 3' ends not matched/total number  
25 of bases in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 bases were perfectly

matched the final percent identity would be 90%. In another example, a 90 base subject sequence is compared with a 100 base query sequence. This time the deletions are internal deletions so that there are no bases on the 5' or 3' of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only bases 5' and 3' of the subject sequence which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are to be made for the purposes of the present invention.

By a polypeptide having an amino acid sequence at least, for example, 95% "identical" to a query amino acid sequence of the present invention, it is intended that the amino acid sequence of the subject polypeptide is identical to the query sequence except that the subject polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the query amino acid sequence. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a query amino acid sequence, up to 5% of the amino acid residues in the subject sequence may be inserted, deleted, (indels) or substituted with another amino acid. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

As a practical matter, whether any particular polypeptide is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to, for instance, an amino acid sequences shown in Table 1 (SEQ ID NO:Y) or to the amino acid sequence encoded by cDNA contained in a deposited clone can be determined conventionally using known computer programs. A preferred method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject

sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag et al. (Comp. App. Biosci. 6:237-245(1990)). In a sequence alignment the query and subject sequences are either both nucleotide sequences or both amino acid sequences. The result of said

5 global sequence alignment is in percent identity. Preferred parameters used in a FASTDB amino acid alignment are: Matrix=PAM 0, k-tuple=2, Mismatch Penalty=1, Joining Penalty=20, Randomization Group Length=0, Cutoff Score=1, Window Size=sequence length, Gap Penalty=5, Gap Size Penalty=0.05, Window Size=500 or the length of the subject amino acid sequence, whichever is shorter.

10 If the subject sequence is shorter than the query sequence due to N- or C-terminal deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for N- and C-terminal truncations of the subject sequence when calculating global percent identity. For subject sequences truncated at the N- and C-termini, relative to the

15 query sequence, the percent identity is corrected by calculating the number of residues of the query sequence that are N- and C-terminal of the subject sequence, which are not matched/aligned with a corresponding subject residue, as a percent of the total bases of the query sequence. Whether a residue is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from

20 the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This final percent identity score is what is used for the purposes of the present invention. Only residues to the N- and C-termini of the subject sequence, which are not matched/aligned with the query sequence, are considered for the purposes of manually adjusting the percent identity

25 score. That is, only query residue positions outside the farthest N- and C-terminal residues of the subject sequence.

For example, a 90 amino acid residue subject sequence is aligned with a 100 residue query sequence to determine percent identity. The deletion occurs at the N-terminus of the subject sequence and therefore, the FASTDB alignment does not show a matching/alignment of the first 10 residues at the N-terminus. The 10  
5 unpaired residues represent 10% of the sequence (number of residues at the N- and C-termini not matched/total number of residues in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 residues were perfectly matched the final percent identity would be 90%. In another example, a 90 residue subject sequence is compared with a 100  
10 residue query sequence. This time the deletions are internal deletions so there are no residues at the N- or C-termini of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only residue positions outside the N- and C-terminal ends of the subject sequence, as displayed in the FASTDB alignment, which are not  
15 matched/aligned with the query sequence are manually corrected for. No other manual corrections are to be made for the purposes of the present invention.

The variants may contain alterations in the coding regions, non-coding regions, or both. Especially preferred are polynucleotide variants containing alterations which produce silent substitutions, additions, or deletions, but do not alter  
20 the properties or activities of the encoded polypeptide. Nucleotide variants produced by silent substitutions due to the degeneracy of the genetic code are preferred. Moreover, variants in which 5-10, 1-5, or 1-2 amino acids are substituted, deleted, or added in any combination are also preferred. Polynucleotide variants can be produced for a variety of reasons, e.g., to optimize codon expression for a particular host  
25 (change codons in the human mRNA to those preferred by a bacterial host such as E. coli).

Naturally occurring variants are called "allelic variants," and refer to one of several alternate forms of a gene occupying a given locus on a chromosome of an organism. (Genes II, Lewin, B., ed., John Wiley & Sons, New York (1985).) These allelic variants can vary at either the polynucleotide and/or polypeptide level and are included in the present invention. Alternatively, non-naturally occurring variants may be produced by mutagenesis techniques or by direct synthesis.

Using known methods of protein engineering and recombinant DNA technology, variants may be generated to improve or alter the characteristics of the polypeptides of the present invention. For instance, one or more amino acids can be deleted from the N-terminus or C-terminus of the secreted protein without substantial loss of biological function. The authors of Ron et al., J. Biol. Chem. 268: 2984-2988 (1993), reported variant KGF proteins having heparin binding activity even after deleting 3, 8, or 27 amino-terminal amino acid residues. Similarly, Interféron gamma exhibited up to ten times higher activity after deleting 8-10 amino acid residues from the carboxy terminus of this protein. (Dobeli et al., J. Biotechnology 7:199-216 (1988).)

Moreover, ample evidence demonstrates that variants often retain a biological activity similar to that of the naturally occurring protein. For example, Gayle and coworkers (J. Biol. Chem 268:22105-22111 (1993)) conducted extensive mutational analysis of human cytokine IL-1a. They used random mutagenesis to generate over 3,500 individual IL-1a mutants that averaged 2.5 amino acid changes per variant over the entire length of the molecule. Multiple mutations were examined at every possible amino acid position. The investigators found that "[m]ost of the molecule could be altered with little effect on either [binding or biological activity]." (See, Abstract.) In fact, only 23 unique amino acid sequences, out of more than 3,500

nucleotide sequences examined, produced a protein that significantly differed in activity from wild-type.

Furthermore, even if deleting one or more amino acids from the N-terminus or C-terminus of a polypeptide results in modification or loss of one or more biological functions, other biological activities may still be retained. For example, the ability of a deletion variant to induce and/or to bind antibodies which recognize the secreted form will likely be retained when less than the majority of the residues of the secreted form are removed from the N-terminus or C-terminus. Whether a particular polypeptide lacking N- or C-terminal residues of a protein retains such immunogenic activities can readily be determined by routine methods described herein and otherwise known in the art.

Thus, the invention further includes polypeptide variants which show substantial biological activity. Such variants include deletions, insertions, inversions, repeats, and substitutions selected according to general rules known in the art so as to have little effect on activity. For example, guidance concerning how to make phenotypically silent amino acid substitutions is provided in Bowie et al., Science 247:1306-1310 (1990), wherein the authors indicate that there are two main strategies for studying the tolerance of an amino acid sequence to change.

The first strategy exploits the tolerance of amino acid substitutions by natural selection during the process of evolution. By comparing amino acid sequences in different species, conserved amino acids can be identified. These conserved amino acids are likely important for protein function. In contrast, the amino acid positions where substitutions have been tolerated by natural selection indicates that these positions are not critical for protein function. Thus, positions tolerating amino acid substitution could be modified while still maintaining biological activity of the protein.

The second strategy uses genetic engineering to introduce amino acid changes at specific positions of a cloned gene to identify regions critical for protein function.

For example, site directed mutagenesis or alanine-scanning mutagenesis (introduction of single alanine mutations at every residue in the molecule) can be used.

- 5 (Cunningham and Wells, Science 244:1081-1085 (1989).) The resulting mutant molecules can then be tested for biological activity.

As the authors state, these two strategies have revealed that proteins are surprisingly tolerant of amino acid substitutions. The authors further indicate which amino acid changes are likely to be permissive at certain amino acid positions in the protein. For example, most buried (within the tertiary structure of the protein) amino acid residues require nonpolar side chains, whereas few features of surface side chains are generally conserved. Moreover, tolerated conservative amino acid substitutions involve replacement of the aliphatic or hydrophobic amino acids Ala, Val, Leu and Ile; replacement of the hydroxyl residues Ser and Thr; replacement of the acidic residues Asp and Glu; replacement of the amide residues Asn and Gln, replacement of the basic residues Lys, Arg, and His; replacement of the aromatic residues Phe, Tyr, and Trp, and replacement of the small-sized amino acids Ala, Ser, Thr, Met, and Gly.

Besides conservative amino acid substitution, variants of the present invention include (i) substitutions with one or more of the non-conserved amino acid residues, where the substituted amino acid residues may or may not be one encoded by the genetic code, or (ii) substitution with one or more of amino acid residues having a substituent group, or (iii) fusion of the mature polypeptide with another compound, such as a compound to increase the stability and/or solubility of the polypeptide (for example, polyethylene glycol), or (iv) fusion of the polypeptide with additional amino acids, such as, for example, an IgG Fc fusion region peptide, or leader or secretory

sequence, or a sequence facilitating purification. Such variant polypeptides are deemed to be within the scope of those skilled in the art from the teachings herein.

For example, polypeptide variants containing amino acid substitutions of charged amino acids with other charged or neutral amino acids may produce proteins with improved characteristics, such as less aggregation. Aggregation of pharmaceutical formulations both reduces activity and increases clearance due to the aggregate's immunogenic activity. (Pinckard et al., Clin. Exp. Immunol. 2:331-340 (1967); Robbins et al., Diabetes 36: 838-845 (1987); Cleland et al., Crit. Rev. Therapeutic Drug Carrier Systems 10:307-377 (1993).)

A further embodiment of the invention relates to a polypeptide which comprises the amino acid sequence of the present invention having an amino acid sequence which contains at least one amino acid substitution, but not more than 50 amino acid substitutions, even more preferably, not more than 40 amino acid substitutions, still more preferably, not more than 30 amino acid substitutions, and still even more preferably, not more than 20 amino acid substitutions. Of course, in order of ever-increasing preference, it is highly preferable for a peptide or polypeptide to have an amino acid sequence which comprises the amino acid sequence of the present invention, which contains at least one, but not more than 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acid substitutions. In specific embodiments, the number of additions, substitutions, and/or deletions in the amino acid sequence of the present invention or fragments thereof (e.g., the mature form and/or other fragments described herein), is 1-5, 5-10, 5-25, 5-50, 10-50 or 50-150, conservative amino acid substitutions are preferable.

## **Polynucleotide and Polypeptide Fragments**



The present invention is also directed to polynucleotide fragments of the polynucleotides of the invention.

In the present invention, a "polynucleotide fragment" refers to a short polynucleotide having a nucleic acid sequence which: is a portion of that contained in a deposited clone, or encoding the polypeptide encoded by the cDNA in a deposited clone; is a portion of that shown in SEQ ID NO:X or the complementary strand thereto, or is a portion of a polynucleotide sequence encoding the polypeptide of SEQ ID NO:Y. The nucleotide fragments of the invention are preferably at least about 15 nt, and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably, at least about 40 nt, at least about 50 nt, at least about 75 nt, or at least about 150 nt in length. A fragment "at least 20 nt in length," for example, is intended to include 20 or more contiguous bases from the cDNA sequence contained in a deposited clone or the nucleotide sequence shown in SEQ ID NO:X. In this context "about" includes the particularly recited value, a value larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini. These nucleotide fragments have uses that include, but are not limited to, as diagnostic probes and primers as discussed herein. Of course, larger fragments (e.g., 50, 150, 500, 600, 2000 nucleotides) are preferred.

Moreover, representative examples of polynucleotide fragments of the invention, include, for example, fragments comprising, or alternatively, consisting of, a sequence from about nucleotide number 1-50, 51-100, 101-150, 151-200, 201-250, 251-300, 301-350, 351-400, 401-450, 451-500, 501-550, 551-600, 651-700, 701-750, 751-800, 800-850, 851-900, 901-950, 951-1000, 1001-1050, 1051-1100, 1101-1150, 1151-1200, 1201-1250, 1251-1300, 1301-1350, 1351-1400, 1401-1450, 1451-1500, 1501-1550, 1551-1600, 1601-1650, 1651-1700, 1701-1750, 1751-1800, 1801-1850, 1851-1900, 1901-1950, 1951-2000, or 2001 to the end of SEQ ID NO:X, or the

complementary strand thereto, or the cDNA contained in a deposited clone. In this context "about" includes the particularly recited ranges, and ranges larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini.

Preferably, these fragments encode a polypeptide which has biological activity. More preferably, these polynucleotides can be used as probes or primers as discussed herein. Polynucleotides which hybridize to these nucleic acid molecules under stringent hybridization conditions or lower stringency conditions are also encompassed by the invention, as are polypeptides encoded by these polynucleotides.

In the present invention, a "polypeptide fragment" refers to an amino acid sequence which is a portion of that contained in SEQ ID NO:Y or encoded by the cDNA contained in a deposited clone. Protein (polypeptide) fragments may be "free-standing," or comprised within a larger polypeptide of which the fragment forms a part or region, most preferably as a single continuous region. Representative examples of polypeptide fragments of the invention, include, for example, fragments comprising, or alternatively consisting of, from about amino acid number 1-20, 21-40, 41-60, 61-80, 81-100, 102-120, 121-140, 141-160, or 161 to the end of the coding region. Moreover, polypeptide fragments can be about 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, or 150 amino acids in length. In this context "about" includes the particularly recited ranges or values, and ranges or values larger or smaller by several (5, 4, 3, 2, or 1) amino acids, at either extreme or at both extremes. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Preferred polypeptide fragments include the secreted protein as well as the mature form. Further preferred polypeptide fragments include the secreted protein or the mature form having a continuous series of deleted residues from the amino or the carboxy terminus, or both. For example, any number of amino acids, ranging from 1-60, can be deleted from the amino terminus of either the secreted polypeptide or the

mature form. Similarly, any number of amino acids, ranging from 1-30, can be deleted from the carboxy terminus of the secreted protein or mature form.

Furthermore, any combination of the above amino and carboxy terminus deletions are preferred. Similarly, polynucleotides encoding these polypeptide fragments are also preferred.

Also preferred are polypeptide and polynucleotide fragments characterized by structural or functional domains, such as fragments that comprise alpha-helix and alpha-helix forming regions, beta-sheet and beta-sheet-forming regions, turn and turn-forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions, substrate binding region, and high antigenic index regions. Polypeptide fragments of SEQ ID NO:Y falling within conserved domains are specifically contemplated by the present invention. Moreover, polynucleotides encoding these domains are also contemplated.

Other preferred polypeptide fragments are biologically active fragments. Biologically active fragments are those exhibiting activity similar, but not necessarily identical, to an activity of the polypeptide of the present invention. The biological activity of the fragments may include an improved desired activity, or a decreased undesirable activity. Polynucleotides encoding these polypeptide fragments are also encompassed by the invention.

Preferably, the polynucleotide fragments of the invention encode a polypeptide which demonstrates a functional activity. By a polypeptide demonstrating a "functional activity" is meant, a polypeptide capable of displaying one or more known functional activities associated with a full-length (complete) polypeptide of invention protein. Such functional activities include, but are not limited to, biological activity, antigenicity [ability to bind (or compete with a

polypeptide of the invention for binding) to an antibody to the polypeptide of the invention], immunogenicity (ability to generate antibody which binds to a polypeptide of the invention), ability to form multimers with polypeptides of the invention, and ability to bind to a receptor or ligand for a polypeptide of the invention.

5           The functional activity of polypeptides of the invention, and fragments, variants derivatives, and analogs thereof, can be assayed by various methods.

For example, in one embodiment where one is assaying for the ability to bind or compete with full-length polypeptide of the invention for binding to an antibody of the polypeptide of the invention, various immunoassays known in the art can be used, including but not limited to, competitive and non-competitive assay systems using techniques such as radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitation reactions, immunodiffusion assays, in situ immunoassays (using colloidal gold, enzyme or radioisotope labels, for example), western blots, precipitation reactions, agglutination assays (e.g., gel agglutination assays, hemagglutination assays), complement fixation assays, immunofluorescence assays, protein A assays, and immunoelectrophoresis assays, etc. In one embodiment, antibody binding is detected by detecting a label on the primary antibody. In another embodiment, the primary antibody is detected by detecting binding of a secondary antibody or reagent to the primary antibody. In a further embodiment, the secondary antibody is labeled. Many means are known in the art for detecting binding in an immunoassay and are within the scope of the present invention.

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In another embodiment, where a ligand for a polypeptide of the invention identified, or the ability of a polypeptide fragment, variant or derivative of the invention to multimerize is being evaluated, binding can be assayed, e.g., by means well-known in the art, such as, for example, reducing and non-reducing gel

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chromatography, protein affinity chromatography, and affinity blotting. See generally, Phizicky, E., et al., 1995, Microbiol. Rev. 59:94-123. In another embodiment, physiological correlates of binding of a polypeptide of the invention to its substrates (signal transduction) can be assayed.

5 In addition, assays described herein (see Examples) and otherwise known in the art may routinely be applied to measure the ability of polypeptides of the invention and fragments, variants derivatives and analogs thereof to elicit related biological activity related to that of the polypeptide of the invention (either in vitro or in vivo). Other methods will be known to the skilled artisan and are within the scope  
10 of the invention.

### Epitopes and Antibodies

The present invention encompasses polypeptides comprising, or alternatively consisting of, an epitope of the polypeptide having an amino acid sequence of SEQ ID  
15 NO:Y, or an epitope of the polypeptide sequence encoded by a polynucleotide sequence contained in ATCC deposit No. Z or encoded by a polynucleotide that hybridizes to the complement of the sequence of SEQ ID NO:X or contained in ATCC deposit No. Z under stringent hybridization conditions or lower stringency hybridization conditions as defined supra. The present invention further encompasses  
20 polynucleotide sequences encoding an epitope of a polypeptide sequence of the invention (such as, for example, the sequence disclosed in SEQ ID NO:X), polynucleotide sequences of the complementary strand of a polynucleotide sequence encoding an epitope of the invention, and polynucleotide sequences which hybridize to the complementary strand under stringent hybridization conditions or lower  
25 stringency hybridization conditions defined supra.

The term "epitopes," as used herein, refers to portions of a polypeptide having antigenic or immunogenic activity in an animal, preferably a mammal, and most preferably in a human. In a preferred embodiment, the present invention encompasses a polypeptide comprising an epitope, as well as the polynucleotide encoding this polypeptide. An "immunogenic epitope," as used herein, is defined as a portion of a protein that elicits an antibody response in an animal, as determined by any method known in the art, for example, by the methods for generating antibodies described *infra*. (See, for example, Geysen et al., Proc. Natl. Acad. Sci. USA 81:3998-4002 (1983)). The term "antigenic epitope," as used herein, is defined as a portion of a protein to which an antibody can immunospecifically bind its antigen as determined by any method well known in the art, for example, by the immunoassays described herein. Immunospecific binding excludes non-specific binding but does not necessarily exclude cross-reactivity with other antigens. Antigenic epitopes need not necessarily be immunogenic.

Fragments which function as epitopes may be produced by any conventional means. (See, e.g., Houghten, Proc. Natl. Acad. Sci. USA 82:5131-5135 (1985), further described in U.S. Patent No. 4,631,211).

In the present invention, antigenic epitopes preferably contain a sequence of at least 4, at least 5, at least 6, at least 7, more preferably at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 20, at least 25, at least 30, at least 40, at least 50, and, most preferably, between about 15 to about 30 amino acids. Preferred polypeptides comprising immunogenic or antigenic epitopes are at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 amino acid residues in length. Additional non-exclusive preferred antigenic epitopes include the antigenic epitopes disclosed herein, as well as portions thereof. Antigenic epitopes are useful, for example, to raise antibodies, including monoclonal antibodies,

that specifically bind the epitope. Preferred antigenic epitopes include the antigenic epitopes disclosed herein, as well as any combination of two, three, four, five or more of these antigenic epitopes. Antigenic epitopes can be used as the target molecules in immunoassays. (See, for instance, Wilson et al., *Cell* 37:767-778 (1984); Sutcliffe et al., *Science* 219:660-666 (1983)).

Similarly, immunogenic epitopes can be used, for example, to induce antibodies according to methods well known in the art. (See, for instance, Sutcliffe et al., *supra*; Wilson et al., *supra*; Chow et al., *Proc. Natl. Acad. Sci. USA* 82:910-914; and Bittle et al., *J. Gen. Virol.* 66:2347-2354 (1985). Preferred immunogenic epitopes include the immunogenic epitopes disclosed herein, as well as any combination of two, three, four, five or more of these immunogenic epitopes. The polypeptides comprising one or more immunogenic epitopes may be presented for eliciting an antibody response together with a carrier protein, such as an albumin, to an animal system (such as rabbit or mouse), or, if the polypeptide is of sufficient length (at least about 25 amino acids), the polypeptide may be presented without a carrier. However, immunogenic epitopes comprising as few as 8 to 10 amino acids have been shown to be sufficient to raise antibodies capable of binding to, at the very least, linear epitopes in a denatured polypeptide (e.g., in Western blotting).

Epitope-bearing polypeptides of the present invention may be used to induce antibodies according to methods well known in the art including, but not limited to, in vivo immunization, in vitro immunization, and phage display methods. See, e.g., Sutcliffe et al., *supra*; Wilson et al., *supra*, and Bittle et al., *J. Gen. Virol.*, 66:2347-2354 (1985). If in vivo immunization is used, animals may be immunized with free peptide; however, anti-peptide antibody titer may be boosted by coupling the peptide to a macromolecular carrier, such as keyhole limpet hemacyanin (KLH) or tetanus toxoid. For instance, peptides containing cysteine residues may be coupled to a

carrier using a linker such as maleimidobenzoyl- N-hydroxysuccinimide ester (MBS), while other peptides may be coupled to carriers using a more general linking agent such as glutaraldehyde. Animals such as rabbits, rats and mice are immunized with either free or carrier- coupled peptides, for instance, by intraperitoneal and/or

5 intradermal injection of emulsions containing about 100  $\mu$ g of peptide or carrier protein and Freund's adjuvant or any other adjuvant known for stimulating an immune response. Several booster injections may be needed, for instance, at intervals of about two weeks, to provide a useful titer of anti-peptide antibody which can be detected, for example, by ELISA assay using free peptide adsorbed to a solid

10 surface. The titer of anti-peptide antibodies in serum from an immunized animal may be increased by selection of anti-peptide antibodies, for instance, by adsorption to the peptide on a solid support and elution of the selected antibodies according to methods well known in the art.

As one of skill in the art will appreciate, and as discussed above, the

15 polypeptides of the present invention comprising an immunogenic or antigenic epitope can be fused to other polypeptide sequences. For example, the polypeptides of the present invention may be fused with the constant domain of immunoglobulins (IgA, IgE, IgG, IgM), or portions thereof (CH1, CH2, CH3, or any combination thereof and portions thereof) resulting in chimeric polypeptides. Such fusion proteins

20 may facilitate purification and may increase half-life in vivo. This has been shown for chimeric proteins consisting of the first two domains of the human CD4- polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. See, e.g., EP 394,827; Traunecker et al., Nature, 331:84-86 (1988). Enhanced delivery of an antigen across the epithelial barrier to the

25 immune system has been demonstrated for antigens (e.g., insulin) conjugated to an FcRn binding partner such as IgG or Fc fragments (see, e.g., PCT Publications WO



96/22024 and WO 99/04813). IgG Fusion proteins that have a disulfide-linked dimeric structure due to the IgG portion desulfide bonds have also been found to be more efficient in binding and neutralizing other molecules than monomeric polypeptides or fragments thereof alone. See, e.g., Fountoulakis et al., J. Biochem., 5 270:3958-3964 (1995). Nucleic acids encoding the above epitopes can also be recombined with a gene of interest as an epitope tag (e.g., the hemagglutinin ("HA") tag or flag tag) to aid in detection and purification of the expressed polypeptide. For example, a system described by Janknecht et al. allows for the ready purification of non-denatured fusion proteins expressed in human cell lines (Janknecht et al., 1991, 10 Proc. Natl. Acad. Sci. USA 88:8972- 897). In this system, the gene of interest is subcloned into a vaccinia recombination plasmid such that the open reading frame of the gene is translationally fused to an amino-terminal tag consisting of six histidine residues. The tag serves as a matrix binding domain for the fusion protein. Extracts from cells infected with the recombinant vaccinia virus are loaded onto Ni<sup>2+</sup> 15 nitriloacetic acid-agarose column and histidine-tagged proteins can be selectively eluted with imidazole-containing buffers.

Additional fusion proteins of the invention may be generated through the techniques of gene-shuffling, motif-shuffling, exon-shuffling, and/or codon-shuffling (collectively referred to as "DNA shuffling"). DNA shuffling may be employed to 20 modulate the activities of polypeptides of the invention, such methods can be used to generate polypeptides with altered activity, as well as agonists and antagonists of the polypeptides. See, generally, U.S. Patent Nos. 5,605,793; 5,811,238; 5,830,721; 5,834,252; and 5,837,458, and Patten et al., Curr. Opinion Biotechnol. 8:724-33 (1997); Harayama, Trends Biotechnol. 16(2):76-82 (1998); Hansson, et al., J. Mol. 25 Biol. 287:265-76 (1999); and Lorenzo and Blasco, Biotechniques 24(2):308- 13 (1998) (each of these patents and publications are hereby incorporated by reference in

its entirety). In one embodiment, alteration of polynucleotides corresponding to SEQ ID NO:X and the polypeptides encoded by these polynucleotides may be achieved by DNA shuffling. DNA shuffling involves the assembly of two or more DNA segments by homologous or site-specific recombination to generate variation in the polynucleotide sequence. In another embodiment, polynucleotides of the invention, or the encoded polypeptides, may be altered by being subjected to random mutagenesis by error-prone PCR, random nucleotide insertion or other methods prior to recombination. In another embodiment, one or more components, motifs, sections, parts, domains, fragments, etc., of a polynucleotide encoding a polypeptide of the invention may be recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous molecules.

#### Antibodies

Further polypeptides of the invention relate to antibodies and T-cell antigen receptors (TCR) which immunospecifically bind a polypeptide, polypeptide fragment, or variant of SEQ ID NO:Y, and/or an epitope, of the present invention (as determined by immunoassays well known in the art for assaying specific antibody-antigen binding). Antibodies of the invention include, but are not limited to, polyclonal, monoclonal, multispecific, human, humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab') fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies (including, e.g., anti-Id antibodies to antibodies of the invention), and epitope-binding fragments of any of the above. The term "antibody," as used herein, refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site that immunospecifically binds an antigen. The immunoglobulin molecules of the invention can be of any type (e.g., IgG, IgE, IgM,

IgD, IgA and IgY), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule.

Most preferably the antibodies are human antigen-binding antibody fragments of the present invention and include, but are not limited to, Fab, Fab' and F(ab')<sub>2</sub>, Fd, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv) and fragments comprising either a VL or VH domain. Antigen-binding antibody fragments, including single-chain antibodies, may comprise the variable region(s) alone or in combination with the entirety or a portion of the following: hinge region, CH1, CH2, and CH3 domains. Also included in the invention are antigen-binding fragments also comprising any combination of variable region(s) with a hinge region, CH1, CH2, and CH3 domains. The antibodies of the invention may be from any animal origin including birds and mammals. Preferably, the antibodies are human, murine (e.g., mouse and rat), donkey, ship rabbit, goat, guinea pig, camel, horse, or chicken. As used herein, "human" antibodies include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies isolated from human immunoglobulin libraries or from animals transgenic for one or more human immunoglobulin and that do not express endogenous immunoglobulins, as described infra and, for example in, U.S. Patent No. 5,939,598 by Kucherlapati et al.

The antibodies of the present invention may be monospecific, bispecific, trispecific or of greater multispecificity. Multispecific antibodies may be specific for different epitopes of a polypeptide of the present invention or may be specific for both a polypeptide of the present invention as well as for a heterologous epitope, such as a heterologous polypeptide or solid support material. See, e.g., PCT publications WO 93/17715; WO 92/08802; WO 91/00360; WO 92/05793; Tutt, et al., J. Immunol. 147:60-69 (1991); U.S. Patent Nos. 4,474,893; 4,714,681; 4,925,648; 5,573,920; 5,601,819; Kostelny et al., J. Immunol. 148:1547-1553 (1992).

Antibodies of the present invention may be described or specified in terms of the epitope(s) or portion(s) of a polypeptide of the present invention which they recognize or specifically bind. The epitope(s) or polypeptide portion(s) may be specified as described herein, e.g., by N-terminal and C-terminal positions, by size in  
5 contiguous amino acid residues, or listed in the Tables and Figures. Antibodies which specifically bind any epitope or polypeptide of the present invention may also be excluded. Therefore, the present invention includes antibodies that specifically bind polypeptides of the present invention, and allows for the exclusion of the same.

Antibodies of the present invention may also be described or specified in  
10 terms of their cross-reactivity. Antibodies that do not bind any other analog, ortholog, or homolog of a polypeptide of the present invention are included. Antibodies that bind polypeptides with at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 65%, at least 60%, at least 55%, and at least 50% identity (as calculated using methods known in the art and described  
15 herein) to a polypeptide of the present invention are also included in the present invention. In specific embodiments, antibodies of the present invention cross-react with murine, rat and/or rabbit homologs of human proteins and the corresponding epitopes thereof. Antibodies that do not bind polypeptides with less than 95%, less than 90%, less than 85%, less than 80%, less than 75%, less than 70%, less than 65%,  
20 less than 60%, less than 55%, and less than 50% identity (as calculated using methods known in the art and described herein) to a polypeptide of the present invention are also included in the present invention. In a specific embodiment, the above-described cross-reactivity is with respect to any single specific antigenic or immunogenic polypeptide, or combination(s) of 2, 3, 4, 5, or more of the specific  
25 antigenic and/or immunogenic polypeptides disclosed herein. Further included in the present invention are antibodies which bind polypeptides encoded by polynucleotides

which hybridize to a polynucleotide of the present invention under stringent hybridization conditions (as described herein). Antibodies of the present invention may also be described or specified in terms of their binding affinity to a polypeptide of the invention. Preferred binding affinities include those with a dissociation constant or  $K_d$  less than  $5 \times 10^{-2}$  M,  $10^{-2}$  M,  $5 \times 10^{-3}$  M,  $10^{-3}$  M,  $5 \times 10^{-4}$  M,  $10^{-4}$  M,  $5 \times 10^{-5}$  M,  $10^{-5}$  M,  $5 \times 10^{-6}$  M,  $10^{-6}$  M,  $5 \times 10^{-7}$  M,  $10^{-7}$  M,  $5 \times 10^{-8}$  M,  $10^{-8}$  M,  $5 \times 10^{-9}$  M,  $10^{-9}$  M,  $5 \times 10^{-10}$  M,  $10^{-10}$  M,  $5 \times 10^{-11}$  M,  $10^{-11}$  M,  $5 \times 10^{-12}$  M,  $10^{-12}$  M,  $5 \times 10^{-13}$  M,  $10^{-13}$  M,  $5 \times 10^{-14}$  M,  $10^{-14}$  M,  $5 \times 10^{-15}$  M, or  $10^{-15}$  M.

The invention also provides antibodies that competitively inhibit binding of an antibody to an epitope of the invention as determined by any method known in the art for determining competitive binding, for example, the immunoassays described herein. In preferred embodiments, the antibody competitively inhibits binding to the epitope by at least 95%, at least 90%, at least 85 %, at least 80%, at least 75%, at least 70%, at least 60%, or at least 50%.

Antibodies of the present invention may act as agonists or antagonists of the polypeptides of the present invention. For example, the present invention includes antibodies which disrupt the receptor/ligand interactions with the polypeptides of the invention either partially or fully. Preferably, antibodies of the present invention bind an antigenic epitope disclosed herein, or a portion thereof. The invention features both receptor-specific antibodies and ligand-specific antibodies. The invention also features receptor-specific antibodies which do not prevent ligand binding but prevent receptor activation. Receptor activation (i.e., signaling) may be determined by techniques described herein or otherwise known in the art. For example, receptor activation can be determined by detecting the phosphorylation (e.g., tyrosine or serine/threonine) of the receptor or its substrate by immunoprecipitation followed by western blot analysis (for example, as described

supra). In specific embodiments, antibodies are provided that inhibit ligand activity or receptor activity by at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, or at least 50% of the activity in absence of the antibody.

- 5           The invention also features receptor-specific antibodies which both prevent ligand binding and receptor activation as well as antibodies that recognize the receptor-ligand complex, and, preferably, do not specifically recognize the unbound receptor or the unbound ligand. Likewise, included in the invention are neutralizing antibodies which bind the ligand and prevent binding of the ligand to the receptor, as
- 10   well as antibodies which bind the ligand, thereby preventing receptor activation, but do not prevent the ligand from binding the receptor. Further included in the invention are antibodies which activate the receptor. These antibodies may act as receptor agonists, i.e., potentiate or activate either all or a subset of the biological activities of the ligand-mediated receptor activation, for example, by inducing dimerization of the
- 15   receptor. The antibodies may be specified as agonists, antagonists or inverse agonists for biological activities comprising the specific biological activities of the peptides of the invention disclosed herein. The above antibody agonists can be made using methods known in the art. See, e.g., PCT publication WO 96/40281; U.S. Patent No. 5,811,097; Deng et al., Blood 92(6):1981-1988 (1998); Chen et al., Cancer Res.
- 20   58(16):3668-3678 (1998); Harrop et al., J. Immunol. 161(4):1786-1794 (1998); Zhu et al., Cancer Res. 58(15):3209-3214 (1998); Yoon et al., J. Immunol. 160(7):3170-3179 (1998); Prat et al., J. Cell. Sci. 111(Pt2):237-247 (1998); Pitard et al., J. Immunol. Methods 205(2):177-190 (1997); Liautard et al., Cytokine 9(4):233-241 (1997); Carlson et al., J. Biol. Chem. 272(17):11295-11301 (1997); Taryman et al.,
- 25   Neuron 14(4):755-762 (1995); Muller et al., Structure 6(9):1153-1167 (1998);

Bartunek et al., Cytokine 8(1):14-20 (1996) (which are all incorporated by reference herein in their entireties).

Antibodies of the present invention may be used, for example, but not limited to, to purify, detect, and target the polypeptides of the present invention, including both in vitro and in vivo diagnostic and therapeutic methods. For example, the antibodies have use in immunoassays for qualitatively and quantitatively measuring levels of the polypeptides of the present invention in biological samples. See, e.g., Harlow et al., Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988) (incorporated by reference herein in its entirety).

As discussed in more detail below, the antibodies of the present invention may be used either alone or in combination with other compositions. The antibodies may further be recombinantly fused to a heterologous polypeptide at the N- or C-terminus or chemically conjugated (including covalently and non-covalently conjugations) to polypeptides or other compositions. For example, antibodies of the present invention may be recombinantly fused or conjugated to molecules useful as labels in detection assays and effector molecules such as heterologous polypeptides, drugs, radionuclides, or toxins. See, e.g., PCT publications WO 92/08495; WO 91/14438; WO 89/12624; U.S. Patent No. 5,314,995; and EP 396,387.

The antibodies of the invention include derivatives that are modified, i.e., by the covalent attachment of any type of molecule to the antibody such that covalent attachment does not prevent the antibody from generating an anti-idiotypic response. For example, but not by way of limitation, the antibody derivatives include antibodies that have been modified, e.g., by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications may be carried out by known techniques,

including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Additionally, the derivative may contain one or more non-classical amino acids.

The antibodies of the present invention may be generated by any suitable method known in the art. Polyclonal antibodies to an antigen-of- interest can be produced by various procedures well known in the art. For example, a polypeptide of the invention can be administered to various host animals including, but not limited to, rabbits, mice, rats, etc. to induce the production of sera containing polyclonal antibodies specific for the antigen. Various adjuvants may be used to increase the immunological response, depending on the host species, and include but are not limited to, Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and corynebacterium parvum. Such adjuvants are also well known in the art.

Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. For example, monoclonal antibodies can be produced using hybridoma techniques including those known in the art and taught, for example, in Harlow et al., *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling, et al., in: *Monoclonal Antibodies and T-Cell Hybridomas* 563-681 (Elsevier, N.Y., 1981) (said references incorporated by reference in their entirety). The term "monoclonal antibody" as used herein is not limited to antibodies produced through hybridoma technology. The term "monoclonal antibody" refers to an antibody that is derived from a single clone,



including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced.

Methods for producing and screening for specific antibodies using hybridoma technology are routine and well known in the art and are discussed in detail in the Examples (e.g., Example 16). In a non-limiting example, mice can be immunized with a polypeptide of the invention or a cell expressing such peptide. Once an immune response is detected, e.g., antibodies specific for the antigen are detected in the mouse serum, the mouse spleen is harvested and splenocytes isolated. The splenocytes are then fused by well known techniques to any suitable myeloma cells, for example cells from cell line SP20 available from the ATCC. Hybridomas are selected and cloned by limited dilution. The hybridoma clones are then assayed by methods known in the art for cells that secrete antibodies capable of binding a polypeptide of the invention. Ascites fluid, which generally contains high levels of antibodies, can be generated by immunizing mice with positive hybridoma clones.

Accordingly, the present invention provides methods of generating monoclonal antibodies as well as antibodies produced by the method comprising culturing a hybridoma cell secreting an antibody of the invention wherein, preferably, the hybridoma is generated by fusing splenocytes isolated from a mouse immunized with an antigen of the invention with myeloma cells and then screening the hybridomas resulting from the fusion for hybridoma clones that secrete an antibody able to bind a polypeptide of the invention.

Antibody fragments which recognize specific epitopes may be generated by known techniques. For example, Fab and F(ab')<sub>2</sub> fragments of the invention may be produced by proteolytic cleavage of immunoglobulin molecules, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')<sub>2</sub> fragments).

F(ab')<sub>2</sub> fragments contain the variable region, the light chain constant region and the CH1 domain of the heavy chain.

For example, the antibodies of the present invention can also be generated using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of phage particles which carry the polynucleotide sequences encoding them. In a particular embodiment, such phage can be utilized to display antigen binding domains expressed from a repertoire or combinatorial antibody library (e.g., human or murine). Phage expressing an antigen binding domain that binds the antigen of interest can be selected or identified with antigen, e.g., using labeled antigen or antigen bound or captured to a solid surface or bead. Phage used in these methods are typically filamentous phage including fd and M13 binding domains expressed from phage with Fab, Fv or disulfide stabilized Fv antibody domains recombinantly fused to either the phage gene III or gene VIII protein. Examples of phage display methods that can be used to make the antibodies of the present invention include those disclosed in Brinkman et al., *J. Immunol. Methods* 182:41-50 (1995); Ames et al., *J. Immunol. Methods* 184:177-186 (1995); Kettleborough et al., *Eur. J. Immunol.* 24:952-958 (1994); Persic et al., *Gene* 187 9-18 (1997); Burton et al., *Advances in Immunology* 57:191-280 (1994); PCT application No. PCT/GB91/01134; PCT publications WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; and U.S. Patent Nos. 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780,225; 5,658,727; 5,733,743 and 5,969,108; each of which is incorporated herein by reference in its entirety.

As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies,

including human antibodies, or any other desired antigen binding fragment, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, e.g., as described in detail below. For example, techniques to recombinantly produce Fab, Fab' and F(ab')<sub>2</sub> fragments can also be employed using  
5 methods known in the art such as those disclosed in PCT publication WO 92/22324; Mullinax et al., *BioTechniques* 12(6):864-869 (1992); and Sawai et al., *AJRI* 34:26-34 (1995); and Better et al., *Science* 240:1041-1043 (1988) (said references incorporated by reference in their entireties).

Examples of techniques which can be used to produce single-chain Fvs and  
10 antibodies include those described in U.S. Patents 4,946,778 and 5,258,498; Huston et al., *Methods in Enzymology* 203:46-88 (1991); Shu et al., *PNAS* 90:7995-7999 (1993); and Skerra et al., *Science* 240:1038-1040 (1988). For some uses, including in vivo use of antibodies in humans and in vitro detection assays, it may be preferable to use chimeric, humanized, or human antibodies. A chimeric antibody is a molecule  
15 in which different portions of the antibody are derived from different animal species, such as antibodies having a variable region derived from a murine monoclonal antibody and a human immunoglobulin constant region. Methods for producing chimeric antibodies are known in the art. See e.g., Morrison, *Science* 229:1202 (1985); Oi et al., *BioTechniques* 4:214 (1986); Gillies et al., (1989) *J. Immunol.*  
20 *Methods* 125:191-202; U.S. Patent Nos. 5,807,715; 4,816,567; and 4,816,397, which are incorporated herein by reference in their entirety. Humanized antibodies are antibody molecules from non-human species antibody that binds the desired antigen having one or more complementarity determining regions (CDRs) from the non-human species and a framework regions from a human immunoglobulin molecule.  
25 Often, framework residues in the human framework regions will be substituted with the corresponding residue from the CDR donor antibody to alter, preferably improve,

antigen binding. These framework substitutions are identified by methods well known in the art, e.g., by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. (See, e.g.,  
5 Queen et al., U.S. Patent No. 5,585,089; Riechmann et al., Nature 332:323 (1988), which are incorporated herein by reference in their entireties.) Antibodies can be humanized using a variety of techniques known in the art including, for example, CDR-grafting (EP 239,400; PCT publication WO 91/09967; U.S. Patent Nos. 5,225,539; 5,530,101; and 5,585,089), veneering or resurfacing (EP 592,106; EP  
10 519,596; Padlan, Molecular Immunology 28(4/5):489-498 (1991); Studnicka et al., Protein Engineering 7(6):805-814 (1994); Roguska. et al., PNAS 91:969-973 (1994)), and chain shuffling (U.S. Patent No. 5,565,332).

Completely human antibodies are particularly desirable for therapeutic treatment of human patients. Human antibodies can be made by a variety of methods  
15 known in the art including phage display methods described above using antibody libraries derived from human immunoglobulin sequences. See also, U.S. Patent Nos. 4,444,887 and 4,716,111; and PCT publications WO 98/46645, WO 98/50433, WO 98/24893, WO 98/16654, WO 96/34096, WO 96/33735, and WO 91/10741; each of which is incorporated herein by reference in its entirety.

20 Human antibodies can also be produced using transgenic mice which are incapable of expressing functional endogenous immunoglobulins, but which can express human immunoglobulin genes. For example, the human heavy and light chain immunoglobulin gene complexes may be introduced randomly or by homologous recombination into mouse embryonic stem cells. Alternatively, the  
25 human variable region, constant region, and diversity region may be introduced into mouse embryonic stem cells in addition to the human heavy and light chain genes.

The mouse heavy and light chain immunoglobulin genes may be rendered non-functional separately or simultaneously with the introduction of human immunoglobulin loci by homologous recombination. In particular, homozygous deletion of the JH region prevents endogenous antibody production. The modified  
5 embryonic stem cells are expanded and microinjected into blastocysts to produce chimeric mice. The chimeric mice are then bred to produce homozygous offspring which express human antibodies. The transgenic mice are immunized in the normal fashion with a selected antigen, e.g., all or a portion of a polypeptide of the invention. Monoclonal antibodies directed against the antigen can be obtained from the  
10 immunized, transgenic mice using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA, IgM and IgE antibodies. For an overview of this technology for producing human  
15 antibodies, see Lonberg and Huszar, *Int. Rev. Immunol.* 13:65-93 (1995). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, see, e.g., PCT publications WO 98/24893; WO 92/01047; WO 96/34096; WO 96/33735; European Patent No. 0 598 877; U.S. Patent Nos. 5,413,923; 5,625,126; 5,633,425; 5,569,825;  
20 5,661,016; 5,545,806; 5,814,318; 5,885,793; 5,916,771; and 5,939,598, which are incorporated by reference herein in their entirety. In addition, companies such as Abgenix, Inc. (Freemont, CA) and Genpharm (San Jose, CA) can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above.

25 Completely human antibodies which recognize a selected epitope can be generated using a technique referred to as "guided selection." In this approach a

selected non-human monoclonal antibody, e.g., a mouse antibody, is used to guide the selection of a completely human antibody recognizing the same epitope. (Jespers et al., Bio/technology 12:899-903 (1988)).

Further, antibodies to the polypeptides of the invention can, in turn, be utilized  
5 to generate anti-idiotypic antibodies that "mimic" polypeptides of the invention using techniques well known to those skilled in the art. (See, e.g., Greenspan & Bona, FASEB J. 7(5):437-444; (1989) and Nissinoff, J. Immunol. 147(8):2429-2438 (1991)). For example, antibodies which bind to and competitively inhibit polypeptide multimerization and/or binding of a polypeptide of the invention to a ligand can be  
10 used to generate anti-idiotypes that "mimic" the polypeptide multimerization and/or binding domain and, as a consequence, bind to and neutralize polypeptide and/or its ligand. Such neutralizing anti-idiotypes or Fab fragments of such anti-idiotypes can be used in therapeutic regimens to neutralize polypeptide ligand. For example, such anti-idiotypic antibodies can be used to bind a polypeptide of the invention and/or to  
15 bind its ligands/receptors, and thereby block its biological activity.

#### Polynucleotides Encoding Antibodies

The invention further provides polynucleotides comprising a nucleotide sequence encoding an antibody of the invention and fragments thereof. The  
20 invention also encompasses polynucleotides that hybridize under stringent or lower stringency hybridization conditions, e.g., as defined supra; to polynucleotides that encode an antibody, preferably, that specifically binds to a polypeptide of the invention, preferably, an antibody that binds to a polypeptide having the amino acid sequence of SEQ ID NO:Y.

25 The polynucleotides may be obtained, and the nucleotide sequence of the polynucleotides determined, by any method known in the art. For example, if the

nucleotide sequence of the antibody is known, a polynucleotide encoding the antibody may be assembled from chemically synthesized oligonucleotides (e.g., as described in Kutmeier et al., *BioTechniques* 17:242 (1994)), which, briefly, involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding the antibody, annealing and ligating of those oligonucleotides, and then amplification of the ligated oligonucleotides by PCR.

Alternatively, a polynucleotide encoding an antibody may be generated from nucleic acid from a suitable source. If a clone containing a nucleic acid encoding a particular antibody is not available, but the sequence of the antibody molecule is known, a nucleic acid encoding the immunoglobulin may be chemically synthesized or obtained from a suitable source (e.g., an antibody cDNA library, or a cDNA library generated from, or nucleic acid, preferably poly A+ RNA, isolated from, any tissue or cells expressing the antibody, such as hybridoma cells selected to express an antibody of the invention) by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence to identify, e.g., a cDNA clone from a cDNA library that encodes the antibody. Amplified nucleic acids generated by PCR may then be cloned into replicable cloning vectors using any method well known in the art.

Once the nucleotide sequence and corresponding amino acid sequence of the antibody is determined, the nucleotide sequence of the antibody may be manipulated using methods well known in the art for the manipulation of nucleotide sequences, e.g., recombinant DNA techniques, site directed mutagenesis, PCR, etc. (see, for example, the techniques described in Sambrook et al., 1990, *Molecular Cloning, A Laboratory Manual*, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY and Ausubel et al., eds., 1998, *Current Protocols in Molecular Biology*, John

Wiley & Sons, NY, which are both incorporated by reference herein in their entireties), to generate antibodies having a different amino acid sequence, for example to create amino acid substitutions, deletions, and/or insertions.

In a specific embodiment, the amino acid sequence of the heavy and/or light chain variable domains may be inspected to identify the sequences of the complementarity determining regions (CDRs) by methods that are well known in the art, e.g., by comparison to known amino acid sequences of other heavy and light chain variable regions to determine the regions of sequence hypervariability. Using routine recombinant DNA techniques, one or more of the CDRs may be inserted within framework regions, e.g., into human framework regions to humanize a non-human antibody, as described supra. The framework regions may be naturally occurring or consensus framework regions, and preferably human framework regions (see, e.g., Chothia et al., J. Mol. Biol. 278: 457-479 (1998) for a listing of human framework regions). Preferably, the polynucleotide generated by the combination of the framework regions and CDRs encodes an antibody that specifically binds a polypeptide of the invention. Preferably, as discussed supra, one or more amino acid substitutions may be made within the framework regions, and, preferably, the amino acid substitutions improve binding of the antibody to its antigen. Additionally, such methods may be used to make amino acid substitutions or deletions of one or more variable region cysteine residues participating in an intrachain disulfide bond to generate antibody molecules lacking one or more intrachain disulfide bonds. Other alterations to the polynucleotide are encompassed by the present invention and within the skill of the art.

In addition, techniques developed for the production of "chimeric antibodies" (Morrison et al., Proc. Natl. Acad. Sci. 81:851-855 (1984); Neuberger et al., Nature 312:604-608 (1984); Takeda et al., Nature 314:452-454 (1985)) by splicing genes



from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. As described supra, a chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived  
5 from a murine mAb and a human immunoglobulin constant region, e.g., humanized antibodies.

Alternatively, techniques described for the production of single chain antibodies (U.S. Patent No. 4,946,778; Bird, Science 242:423- 42 (1988); Huston et al., Proc. Natl. Acad. Sci. USA 85:5879-5883 (1988); and Ward et al., Nature  
10 334:544-54 (1989)) can be adapted to produce single chain antibodies. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide. Techniques for the assembly of functional Fv fragments in E. coli may also be used (Skerra et al., Science 242:1038- 1041 (1988)).

15

#### Methods of Producing Antibodies

The antibodies of the invention can be produced by any method known in the art for the synthesis of antibodies, in particular, by chemical synthesis or preferably, by recombinant expression techniques.

20

Recombinant expression of an antibody of the invention, or fragment, derivative or analog thereof, (e.g., a heavy or light chain of an antibody of the invention or a single chain antibody of the invention), requires construction of an expression vector containing a polynucleotide that encodes the antibody. Once a polynucleotide encoding an antibody molecule or a heavy or light chain of an  
25 antibody, or portion thereof (preferably containing the heavy or light chain variable domain), of the invention has been obtained, the vector for the production of the

antibody molecule may be produced by recombinant DNA technology using techniques well known in the art. Thus, methods for preparing a protein by expressing a polynucleotide containing an antibody encoding nucleotide sequence are described herein. Methods which are well known to those skilled in the art can be used to construct expression vectors containing antibody coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. The invention, thus, provides replicable vectors comprising a nucleotide sequence encoding an antibody molecule of the invention, or a heavy or light chain thereof, or a heavy or light chain variable domain, operably linked to a promoter. Such vectors may include the nucleotide sequence encoding the constant region of the antibody molecule (see, e.g., PCT Publication WO 86/05807; PCT Publication WO 89/01036; and U.S. Patent No. 5,122,464) and the variable domain of the antibody may be cloned into such a vector for expression of the entire heavy or light chain.

The expression vector is transferred to a host cell by conventional techniques and the transfected cells are then cultured by conventional techniques to produce an antibody of the invention. Thus, the invention includes host cells containing a polynucleotide encoding an antibody of the invention, or a heavy or light chain thereof, or a single chain antibody of the invention, operably linked to a heterologous promoter. In preferred embodiments for the expression of double-chained antibodies, vectors encoding both the heavy and light chains may be co-expressed in the host cell for expression of the entire immunoglobulin molecule, as detailed below.

A variety of host-expression vector systems may be utilized to express the antibody molecules of the invention. Such host-expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently

purified, but also represent cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, express an antibody molecule of the invention in situ. These include but are not limited to microorganisms such as bacteria (e.g., *E. coli*, *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing antibody coding sequences; yeast (e.g., *Saccharomyces*, *Pichia*) transformed with recombinant yeast expression vectors containing antibody coding sequences; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing antibody coding sequences; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing antibody coding sequences; or mammalian cell systems (e.g., COS, CHO, BHK, 293, 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia virus 7.5K promoter). Preferably, bacterial cells such as *Escherichia coli*, and more preferably, eukaryotic cells, especially for the expression of whole recombinant antibody molecule, are used for the expression of a recombinant antibody molecule. For example, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for antibodies (Foecking et al., *Gene* 45:101 (1986); Cockett et al., *Bio/Technology* 8:2 (1990)).

In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the antibody molecule being expressed. For example, when a large quantity of such a protein is to be produced, for the

generation of pharmaceutical compositions of an antibody molecule, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited, to the *E. coli* expression vector pUR278 (Ruther et al., EMBO J. 2:1791 (1983)), in which the antibody coding sequence may be ligated individually into the vector in frame with the lac Z coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, Nucleic Acids Res. 13:3101-3109 (1985); Van Heeke & Schuster, J. Biol. Chem. 24:5503-5509 (1989)); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption and binding to matrix glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

15 In an insect system, Autographa californica nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The antibody coding sequence may be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter).

20 In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the antibody coding sequence of interest may be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by in vitro or in vivo recombination. Insertion in a non-essential region of the viral genome (e.g., region

E1 or E3) will result in a recombinant virus that is viable and capable of expressing the antibody molecule in infected hosts. (e.g., see Logan & Shenk, Proc. Natl. Acad. Sci. USA 81:355-359 (1984)). Specific initiation signals may also be required for efficient translation of inserted antibody coding sequences. These signals include the

5 ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate

10 transcription enhancer elements, transcription terminators, etc. (see Bittner et al., Methods in Enzymol. 153:51-544 (1987)).

In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (e.g., glycosylation) and processing (e.g.,

15 cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells which

20 possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include but are not limited to CHO, VERY, BHK, Hela, COS, MDCK, 293, 3T3, WI38, and in particular, breast cancer cell lines such as, for example, BT483, Hs578T, HTB2, BT20 and T47D, and normal mammary gland cell

25 line such as, for example, CRL7030 and Hs578Bst.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express the antibody molecule may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (e.g., promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines which express the antibody molecule. Such engineered cell lines may be particularly useful in screening and evaluation of compounds that interact directly or indirectly with the antibody molecule.

A number of selection systems may be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler et al., Cell 11:223 (1977)), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, Proc. Natl. Acad. Sci. USA 48:202 (1992)), and adenine phosphoribosyltransferase (Lowy et al., Cell 22:817 (1980)) genes can be employed in tk-, hgp<sup>r</sup>t- or ap<sup>r</sup>t- cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: dhfr, which confers resistance to methotrexate (Wigler et al., Natl. Acad. Sci. USA 77:357 (1980); O'Hare et al., Proc. Natl. Acad. Sci. USA 78:1527 (1981)); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, Proc. Natl. Acad. Sci. USA 78:2072 (1981)); neo, which confers resistance to the aminoglycoside G-418 Clinical Pharmacy 12:488-505; Wu and Wu, Biotherapy 3:87-95 (1991); Tolstoshev, Ann. Rev. Pharmacol. Toxicol. 32:573-596 (1993); Mulligan, Science

260:926-932 (1993); and Morgan and Anderson, *Ann. Rev. Biochem.* 62:191-217. (1993); May, 1993, *TIB TECH* 11(5):155-215); and hygromycin, which confers resistance to hygromycin (Santerre et al., *Gene* 30:147 (1984)). Methods commonly known in the art of recombinant DNA technology may be routinely applied to select the desired recombinant clone, and such methods are described, for example, in Ausubel et al. (eds.), *Current Protocols in Molecular Biology*, John Wiley & Sons, NY (1993); Kriegler, *Gene Transfer and Expression, A Laboratory Manual*, Stockton Press, NY (1990); and in Chapters 12 and 13, Dracopoli et al. (eds), *Current Protocols in Human Genetics*, John Wiley & Sons, NY (1994); Colberre-Garapin et al., *J. Mol. Biol.* 150:1 (1981), which are incorporated by reference herein in their entireties.

The expression levels of an antibody molecule can be increased by vector amplification (for a review, see Bebbington and Hentschel, *The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning*, Vol.3. (Academic Press, New York, 1987)). When a marker in the vector system expressing antibody is amplifiable, increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the antibody gene, production of the antibody will also increase (Crouse et al., *Mol. Cell. Biol.* 3:257 (1983)).

The host cell may be co-transfected with two expression vectors of the invention, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide. The two vectors may contain identical selectable markers which enable equal expression of heavy and light chain polypeptides. Alternatively, a single vector may be used which encodes, and is capable of expressing, both heavy and light chain polypeptides. In such situations, the light chain should be placed before the heavy chain to avoid an excess of toxic free heavy chain (Proudfoot, *Nature* 322:52 (1986); Kohler, *Proc. Natl. Acad. Sci.*

USA 77:2197 (1980)). The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA.

Once an antibody molecule of the invention has been produced by an animal, chemically synthesized, or recombinantly expressed, it may be purified by any  
5 method known in the art for purification of an immunoglobulin molecule, for example, by chromatography (e.g., ion exchange, affinity, particularly by affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. In addition, the antibodies of the present invention or  
10 fragments thereof can be fused to heterologous polypeptide sequences described herein or otherwise known in the art, to facilitate purification.

The present invention encompasses antibodies recombinantly fused or chemically conjugated (including both covalently and non-covalently conjugations) to a polypeptide (or portion thereof, preferably at least 10, 20, 30, 40, 50, 60, 70, 80,  
15 90 or 100 amino acids of the polypeptide) of the present invention to generate fusion proteins. The fusion does not necessarily need to be direct, but may occur through linker sequences. The antibodies may be specific for antigens other than polypeptides (or portion thereof, preferably at least 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 amino acids of the polypeptide) of the present invention. For example, antibodies may be  
20 used to target the polypeptides of the present invention to particular cell types, either in vitro or in vivo, by fusing or conjugating the polypeptides of the present invention to antibodies specific for particular cell surface receptors. Antibodies fused or conjugated to the polypeptides of the present invention may also be used in in vitro immunoassays and purification methods using methods known in the art. See e.g.,  
25 Harbor et al., supra, and PCT publication WO 93/21232; EP 439,095; Naramura et al., Immunol. Lett. 39:91-99 (1994); U.S. Patent 5,474,981; Gillies et al., PNAS



89:1428-1432 (1992); Fell et al., J. Immunol. 146:2446-2452(1991), which are incorporated by reference in their entireties.

The present invention further includes compositions comprising the polypeptides of the present invention fused or conjugated to antibody domains other than the variable regions. For example, the polypeptides of the present invention may be fused or conjugated to an antibody Fc region, or portion thereof. The antibody portion fused to a polypeptide of the present invention may comprise the constant region, hinge region, CH1 domain, CH2 domain, and CH3 domain or any combination of whole domains or portions thereof. The polypeptides may also be fused or conjugated to the above antibody portions to form multimers. For example, Fc portions fused to the polypeptides of the present invention can form dimers through disulfide bonding between the Fc portions. Higher multimeric forms can be made by fusing the polypeptides to portions of IgA and IgM. Methods for fusing or conjugating the polypeptides of the present invention to antibody portions are known in the art. See, e.g., U.S. Patent Nos. 5,336,603; 5,622,929; 5,359,046; 5,349,053; 5,447,851; 5,112,946; EP 307,434; EP 367,166; PCT publications WO 96/04388; WO 91/06570; Ashkenazi et al., Proc. Natl. Acad. Sci. USA 88:10535-10539 (1991); Zheng et al., J. Immunol. 154:5590-5600 (1995); and Vil et al., Proc. Natl. Acad. Sci. USA 89:11337- 11341(1992) (said references incorporated by reference in their entireties).

As discussed, supra, the polypeptides corresponding to a polypeptide, polypeptide fragment, or a variant of SEQ ID NO:Y may be fused or conjugated to the above antibody portions to increase the in vivo half life of the polypeptides or for use in immunoassays using methods known in the art. Further, the polypeptides corresponding to SEQ ID NO:Y may be fused or conjugated to the above antibody portions to facilitate purification. One reported example describes chimeric proteins

consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. (EP 394,827; Traunecker et al., *Nature* 331:84-86 (1988)). The polypeptides of the present invention fused or conjugated to an antibody having

5 disulfide-linked dimeric structures (due to the IgG) may also be more efficient in binding and neutralizing other molecules, than the monomeric secreted protein or protein fragment alone. (Fountoulakis et al., *J. Biochem.* 270:3958-3964 (1995)). In many cases, the Fc part in a fusion protein is beneficial in therapy and diagnosis, and thus can result in, for example, improved pharmacokinetic properties. (EP A

10 232,262). Alternatively, deleting the Fc part after the fusion protein has been expressed, detected, and purified, would be desired. For example, the Fc portion may hinder therapy and diagnosis if the fusion protein is used as an antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to

15 identify antagonists of hIL-5. (See, Bennett et al., *J. Molecular Recognition* 8:52-58 (1995); Johanson et al., *J. Biol. Chem.* 270:9459-9471 (1995)).

Moreover, the antibodies or fragments thereof of the present invention can be fused to marker sequences, such as a peptide to facilitate purification. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the

20 tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz et al., *Proc. Natl. Acad. Sci. USA* 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Other peptide tags useful for purification include, but are not limited to, the "HA" tag, which

25 corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson et al., *Cell* 37:767 (1984)) and the "flag" tag.

The present invention further encompasses antibodies or fragments thereof conjugated to a diagnostic or therapeutic agent. The antibodies can be used diagnostically to, for example, monitor the development or progression of a tumor as part of a clinical testing procedure to, e.g., determine the efficacy of a given

5 treatment regimen. Detection can be facilitated by coupling the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, radioactive materials, positron emitting metals using various positron emission tomographies, and nonradioactive paramagnetic metal ions. The detectable

10 substance may be coupled or conjugated either directly to the antibody (or fragment thereof) or indirectly, through an intermediate (such as, for example, a linker known in the art) using techniques known in the art. See, for example, U.S. Patent No. 4,741,900 for metal ions which can be conjugated to antibodies for use as diagnostics according to the present invention. Examples of suitable enzymes include horseradish

15 peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material

20 includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin; and examples of suitable radioactive material include  $^{125}\text{I}$ ,  $^{131}\text{I}$ ,  $^{111}\text{In}$  or  $^{99}\text{Tc}$ .

Further, an antibody or fragment thereof may be conjugated to a therapeutic moiety such as a cytotoxin, e.g., a cytostatic or cytotoxic agent, a therapeutic agent or

25 a radioactive metal ion, e.g., alpha-emitters such as, for example,  $^{213}\text{Bi}$ . A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include

paclitaxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (e.g., vincristine and vinblastine).

The conjugates of the invention can be used for modifying a given biological response, the therapeutic agent or drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor,  $\alpha$ -interferon,  $\beta$ -interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator, an apoptotic agent, e.g., TNF- $\alpha$ , TNF- $\beta$ , AIM I (See, International Publication No. WO 97/33899), AIM II (See, International Publication No. WO 97/34911), Fas Ligand (Takahashi *et al.*, *Int. Immunol.*, 6:1567-1574 (1994)), VEGF (See, International Publication No. WO 99/23105), a thrombotic agent or an anti-angiogenic agent, e.g., angiostatin or endostatin; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"),

granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

Antibodies may also be attached to solid supports, which are particularly useful for immunoassays or purification of the target antigen. Such solid supports  
5 include, but are not limited to, glass, cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene.

Techniques for conjugating such therapeutic moiety to antibodies are well known, see, e.g., Arnon et al., "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in *Monoclonal Antibodies And Cancer Therapy*, Reisfeld  
10 et al. (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom et al., "Antibodies For Drug Delivery", in *Controlled Drug Delivery* (2nd Ed.), Robinson et al. (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in *Monoclonal Antibodies '84: Biological And Clinical Applications*, Pinchera et al. (eds.), pp. 475-506 (1985); "Analysis, Results,  
15 And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in *Monoclonal Antibodies For Cancer Detection And Therapy*, Baldwin et al. (eds.), pp. 303-16 (Academic Press 1985), and Thorpe et al., "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", *Immunol. Rev.* 62:119-58 (1982).

20 Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980, which is incorporated herein by reference in its entirety.

An antibody, with or without a therapeutic moiety conjugated to it, administered alone or in combination with cytotoxic factor(s) and/or cytokine(s) can  
25 be used as a therapeutic.

### Immunophenotyping

The antibodies of the invention may be utilized for immunophenotyping of cell lines and biological samples. The translation product of the gene of the present invention may be useful as a cell specific marker, or more specifically as a cellular  
5 marker that is differentially expressed at various stages of differentiation and/or maturation of particular cell types. Monoclonal antibodies directed against a specific epitope, or combination of epitopes, will allow for the screening of cellular populations expressing the marker. Various techniques can be utilized using monoclonal antibodies to screen for cellular populations expressing the marker(s), and  
10 include magnetic separation using antibody-coated magnetic beads, "panning" with antibody attached to a solid matrix (i.e., plate), and flow cytometry (See, e.g., U.S. Patent 5,985,660; and Morrison *et al.*, *Cell*, 96:737-49 (1999)).

These techniques allow for the screening of particular populations of cells, such as might be found with hematological malignancies (i.e. minimal residual  
15 disease (MRD) in acute leukemic patients) and "non-self" cells in transplantations to prevent Graft-versus-Host Disease (GVHD). Alternatively, these techniques allow for the screening of hematopoietic stem and progenitor cells capable of undergoing proliferation and/or differentiation, as might be found in human umbilical cord blood.

### 20 Assays For Antibody Binding

The antibodies of the invention may be assayed for immunospecific binding by any method known in the art. The immunoassays which can be used include but are not limited to competitive and non-competitive assay systems using techniques such as western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent  
25 assay), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays,

complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, protein A immunoassays, to name but a few. Such assays are routine and well known in the art (see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York, which is incorporated by  
5 reference herein in its entirety). Exemplary immunoassays are described briefly below (but are not intended by way of limitation).

Immunoprecipitation protocols generally comprise lysing a population of cells in a lysis buffer such as RIPA buffer (1% NP-40 or Triton X- 100, 1% sodium deoxycholate, 0.1% SDS, 0.15 M NaCl, 0.01 M sodium phosphate at pH 7.2, 1%  
10 Trasylol) supplemented with protein phosphatase and/or protease inhibitors (e.g., EDTA, PMSF, aprotinin, sodium vanadate), adding the antibody of interest to the cell lysate, incubating for a period of time (e.g., 1-4 hours) at 4° C, adding protein A and/or protein G sepharose beads to the cell lysate, incubating for about an hour or more at 4° C, washing the beads in lysis buffer and resuspending the beads in  
15 SDS/sample buffer. The ability of the antibody of interest to immunoprecipitate a particular antigen can be assessed by, e.g., western blot analysis. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the binding of the antibody to an antigen and decrease the background (e.g., pre-clearing the cell lysate with sepharose beads). For further discussion regarding  
20 immunoprecipitation protocols see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.16.1.

Western blot analysis generally comprises preparing protein samples, electrophoresis of the protein samples in a polyacrylamide gel (e.g., 8%- 20% SDS-PAGE depending on the molecular weight of the antigen), transferring the protein  
25 sample from the polyacrylamide gel to a membrane such as nitrocellulose, PVDF or nylon, blocking the membrane in blocking solution (e.g., PBS with 3% BSA or non-

fat milk), washing the membrane in washing buffer (e.g., PBS-Tween 20), blocking the membrane with primary antibody (the antibody of interest) diluted in blocking buffer, washing the membrane in washing buffer, blocking the membrane with a secondary antibody (which recognizes the primary antibody, e.g., an anti-human  
5 antibody) conjugated to an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) or radioactive molecule (e.g., <sup>32</sup>P or <sup>125</sup>I) diluted in blocking buffer, washing the membrane in wash buffer, and detecting the presence of the antigen. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected and to reduce the background noise. For  
10 further discussion regarding western blot protocols see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.8.1.

ELISAs comprise preparing antigen, coating the well of a 96 well microtiter plate with the antigen, adding the antibody of interest conjugated to a detectable  
15 compound such as an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) to the well and incubating for a period of time, and detecting the presence of the antigen. In ELISAs the antibody of interest does not have to be conjugated to a detectable compound; instead, a second antibody (which recognizes the antibody of interest) conjugated to a detectable compound may be added to the  
20 well. Further, instead of coating the well with the antigen, the antibody may be coated to the well. In this case, a second antibody conjugated to a detectable compound may be added following the addition of the antigen of interest to the coated well. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected as well as other variations of ELISAs  
25 known in the art. For further discussion regarding ELISAs see, e.g., Ausubel et al,



eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 11.2.1.

The binding affinity of an antibody to an antigen and the off-rate of an antibody-antigen interaction can be determined by competitive binding assays. One  
5 example of a competitive binding assay is a radioimmunoassay comprising the incubation of labeled antigen (e.g.,  $^3\text{H}$  or  $^{125}\text{I}$ ) with the antibody of interest in the presence of increasing amounts of unlabeled antigen, and the detection of the antibody bound to the labeled antigen. The affinity of the antibody of interest for a particular antigen and the binding off-rates can be determined from the data by  
10 scatchard plot analysis. Competition with a second antibody can also be determined using radioimmunoassays. In this case, the antigen is incubated with antibody of interest conjugated to a labeled compound (e.g.,  $^3\text{H}$  or  $^{125}\text{I}$ ) in the presence of increasing amounts of an unlabeled second antibody.

#### 15      Therapeutic Uses

The present invention is further directed to antibody-based therapies which involve administering antibodies of the invention to an animal, preferably a mammal, and most preferably a human, patient for treating one or more of the disclosed diseases, disorders, or conditions. Therapeutic compounds of the invention include,  
20 but are not limited to, antibodies of the invention (including fragments, analogs and derivatives thereof as described herein) and nucleic acids encoding antibodies of the invention (including fragments, analogs and derivatives thereof and anti-idiotypic antibodies as described herein). The antibodies of the invention can be used to treat, inhibit or prevent diseases, disorders or conditions associated with aberrant expression  
25 and/or activity of a polypeptide of the invention, including, but not limited to, any one or more of the diseases, disorders, or conditions described herein. The treatment

and/or prevention of diseases, disorders, or conditions associated with aberrant expression and/or activity of a polypeptide of the invention includes, but is not limited to, alleviating symptoms associated with those diseases, disorders or conditions. Antibodies of the invention may be provided in pharmaceutically acceptable compositions as known in the art or as described herein.

A summary of the ways in which the antibodies of the present invention may be used therapeutically includes binding polynucleotides or polypeptides of the present invention locally or systemically in the body or by direct cytotoxicity of the antibody, e.g. as mediated by complement (CDC) or by effector cells (ADCC).

Some of these approaches are described in more detail below. Armed with the teachings provided herein, one of ordinary skill in the art will know how to use the antibodies of the present invention for diagnostic, monitoring or therapeutic purposes without undue experimentation.

The antibodies of this invention may be advantageously utilized in combination with other monoclonal or chimeric antibodies, or with lymphokines or hematopoietic growth factors (such as, e.g., IL-2, IL-3 and IL-7), for example, which serve to increase the number or activity of effector cells which interact with the antibodies.

The antibodies of the invention may be administered alone or in combination with other types of treatments (e.g., radiation therapy, chemotherapy, hormonal therapy, immunotherapy and anti-tumor agents). Generally, administration of products of a species origin or species reactivity (in the case of antibodies) that is the same species as that of the patient is preferred. Thus, in a preferred embodiment, human antibodies, fragments derivatives, analogs, or nucleic acids, are administered to a human patient for therapy or prophylaxis.

It is preferred to use high affinity and/or potent in vivo inhibiting and/or neutralizing antibodies against polypeptides or polynucleotides of the present invention, fragments or regions thereof, for both immunoassays directed to and therapy of disorders related to polynucleotides or polypeptides, including fragments thereof, of the present invention. Such antibodies, fragments, or regions, will preferably have an affinity for polynucleotides or polypeptides of the invention, including fragments thereof. Preferred binding affinities include those with a dissociation constant or  $K_d$  less than  $5 \times 10^{-2}$  M,  $10^{-2}$  M,  $5 \times 10^{-3}$  M,  $10^{-3}$  M,  $5 \times 10^{-4}$  M,  $10^{-4}$  M,  $5 \times 10^{-5}$  M,  $10^{-5}$  M,  $5 \times 10^{-6}$  M,  $10^{-6}$  M,  $5 \times 10^{-7}$  M,  $10^{-7}$  M,  $5 \times 10^{-8}$  M,  $10^{-8}$  M,  $5 \times 10^{-9}$  M,  $10^{-9}$  M,  $5 \times 10^{-10}$  M,  $10^{-10}$  M,  $5 \times 10^{-11}$  M,  $10^{-11}$  M,  $5 \times 10^{-12}$  M,  $10^{-12}$  M,  $5 \times 10^{-13}$  M,  $10^{-13}$  M,  $5 \times 10^{-14}$  M,  $10^{-14}$  M,  $5 \times 10^{-15}$  M, and  $10^{-15}$  M.

#### Gene Therapy

In a specific embodiment, nucleic acids comprising sequences encoding antibodies or functional derivatives thereof, are administered to treat, inhibit or prevent a disease or disorder associated with aberrant expression and/or activity of a polypeptide of the invention, by way of gene therapy. Gene therapy refers to therapy performed by the administration to a subject of an expressed or expressible nucleic acid. In this embodiment of the invention, the nucleic acids produce their encoded protein that mediates a therapeutic effect.

Any of the methods for gene therapy available in the art can be used according to the present invention. Exemplary methods are described below.

For general reviews of the methods of gene therapy, see Goldspiel et al., Clinical Pharmacy 12:488-505 (1993); Wu and Wu, Biotherapy 3:87-95 (1991); Tolstoshev, Ann. Rev. Pharmacol. Toxicol. 32:573-596 (1993); Mulligan, Science 260:926-932 (1993); and Morgan and Anderson, Ann. Rev. Biochem. 62:191-217

(1993); May, TIBTECH 11(5):155-215 (1993). Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); and Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY  
5 (1990).

In a preferred aspect, the compound comprises nucleic acid sequences encoding an antibody, said nucleic acid sequences being part of expression vectors that express the antibody or fragments or chimeric proteins or heavy or light chains thereof in a suitable host. In particular, such nucleic acid sequences have promoters  
10 operably linked to the antibody coding region, said promoter being inducible or constitutive, and, optionally, tissue- specific. In another particular embodiment, nucleic acid molecules are used in which the antibody coding sequences and any other desired sequences are flanked by regions that promote homologous recombination at a desired site in the genome, thus providing for intrachromosomal expression of the  
15 antibody encoding nucleic acids (Koller and Smithies, Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); Zijlstra et al., Nature 342:435-438 (1989). In specific embodiments, the expressed antibody molecule is a single chain antibody; alternatively, the nucleic acid sequences include sequences encoding both the heavy and light chains, or fragments thereof, of the antibody.

20 Delivery of the nucleic acids into a patient may be either direct, in which case the patient is directly exposed to the nucleic acid or nucleic acid- carrying vectors, or indirect, in which case, cells are first transformed with the nucleic acids in vitro, then transplanted into the patient. These two approaches are known, respectively, as in vivo or ex vivo gene therapy.

25 In a specific embodiment, the nucleic acid sequences are directly administered in vivo, where it is expressed to produce the encoded product. This can be

accomplished by any of numerous methods known in the art, e.g., by constructing them as part of an appropriate nucleic acid expression vector and administering it so that they become intracellular, e.g., by infection using defective or attenuated retrovirals or other viral vectors (see U.S. Patent No. 4,980,286), or by direct  
5 injection of naked DNA, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, encapsulation in liposomes, microparticles, or microcapsules, or by administering them in linkage to a peptide which is known to enter the nucleus, by administering it in linkage to a ligand subject to receptor-mediated endocytosis (see,  
10 e.g., Wu and Wu, J. Biol. Chem. 262:4429-4432 (1987)) (which can be used to target cell types specifically expressing the receptors), etc. In another embodiment, nucleic acid-ligand complexes can be formed in which the ligand comprises a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. In yet another embodiment, the nucleic acid can be targeted in vivo for  
15 cell specific uptake and expression, by targeting a specific receptor (see, e.g., PCT Publications WO 92/06180; WO 92/22635; WO92/20316; WO93/14188, WO 93/20221). Alternatively, the nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination (Koller and Smithies, Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); Zijlstra et al.,  
20 Nature 342:435-438 (1989)).

In a specific embodiment, viral vectors that contains nucleic acid sequences encoding an antibody of the invention are used. For example, a retroviral vector can be used (see Miller et al., Meth. Enzymol. 217:581-599 (1993)). These retroviral vectors contain the components necessary for the correct packaging of the viral  
25 genome and integration into the host cell DNA. The nucleic acid sequences encoding the antibody to be used in gene therapy are cloned into one or more vectors, which

facilitates delivery of the gene into a patient. More detail about retroviral vectors can be found in Boesen et al., *Biotherapy* 6:291-302 (1994), which describes the use of a retroviral vector to deliver the *mdr1* gene to hematopoietic stem cells in order to make the stem cells more resistant to chemotherapy. Other references illustrating the use of retroviral vectors in gene therapy are: Clowes et al., *J. Clin. Invest.* 93:644-651 (1994); Kiem et al., *Blood* 83:1467-1473 (1994); Salmons and Gunzberg, *Human Gene Therapy* 4:129-141 (1993); and Grossman and Wilson, *Curr. Opin. in Genetics and Devel.* 3:110-114 (1993).

Adenoviruses are other viral vectors that can be used in gene therapy.

Adenoviruses are especially attractive vehicles for delivering genes to respiratory epithelia. Adenoviruses naturally infect respiratory epithelia where they cause a mild disease. Other targets for adenovirus-based delivery systems are liver, the central nervous system, endothelial cells, and muscle. Adenoviruses have the advantage of being capable of infecting non-dividing cells. Kozarsky and Wilson, *Current Opinion in Genetics and Development* 3:499-503 (1993) present a review of adenovirus-based gene therapy. Bout et al., *Human Gene Therapy* 5:3-10 (1994) demonstrated the use of adenovirus vectors to transfer genes to the respiratory epithelia of rhesus monkeys. Other instances of the use of adenoviruses in gene therapy can be found in Rosenfeld et al., *Science* 252:431-434 (1991); Rosenfeld et al., *Cell* 68:143-155 (1992); Mastrangeli et al., *J. Clin. Invest.* 91:225-234 (1993); PCT Publication WO94/12649; and Wang, et al., *Gene Therapy* 2:775-783 (1995). In a preferred embodiment, adenovirus vectors are used.

Adeno-associated virus (AAV) has also been proposed for use in gene therapy (Walsh et al., *Proc. Soc. Exp. Biol. Med.* 204:289-300 (1993); U.S. Patent No. 5,436,146).

Another approach to gene therapy involves transferring a gene to cells in tissue culture by such methods as electroporation, lipofection, calcium phosphate mediated transfection, or viral infection. Usually, the method of transfer includes the transfer of a selectable marker to the cells. The cells are then placed under selection to isolate those cells that have taken up and are expressing the transferred gene. Those cells are then delivered to a patient.

In this embodiment, the nucleic acid is introduced into a cell prior to administration in vivo of the resulting recombinant cell. Such introduction can be carried out by any method known in the art, including but not limited to transfection, electroporation, microinjection, infection with a viral or bacteriophage vector containing the nucleic acid sequences, cell fusion, chromosome-mediated gene transfer, microcell-mediated gene transfer, spheroplast fusion, etc. Numerous techniques are known in the art for the introduction of foreign genes into cells (see, e.g., Loeffler and Behr, Meth. Enzymol. 217:599-618 (1993); Cohen et al., Meth. Enzymol. 217:618-644 (1993); Cline, Pharmac. Ther. 29:69-92m (1985) and may be used in accordance with the present invention, provided that the necessary developmental and physiological functions of the recipient cells are not disrupted. The technique should provide for the stable transfer of the nucleic acid to the cell, so that the nucleic acid is expressible by the cell and preferably heritable and expressible by its cell progeny.

The resulting recombinant cells can be delivered to a patient by various methods known in the art. Recombinant blood cells (e.g., hematopoietic stem or progenitor cells) are preferably administered intravenously. The amount of cells envisioned for use depends on the desired effect, patient state, etc., and can be determined by one skilled in the art.

Cells into which a nucleic acid can be introduced for purposes of gene therapy encompass any desired, available cell type, and include but are not limited to epithelial cells, endothelial cells, keratinocytes, fibroblasts, muscle cells, hepatocytes; blood cells such as Tlymphocytes, Blymphocytes, monocytes, macrophages, neutrophils, eosinophils, megakaryocytes, granulocytes; various stem or progenitor cells, in particular hematopoietic stem or progenitor cells, e.g., as obtained from bone marrow, umbilical cord blood, peripheral blood, fetal liver, etc.

In a preferred embodiment, the cell used for gene therapy is autologous to the patient.

10 In an embodiment in which recombinant cells are used in gene therapy, nucleic acid sequences encoding an antibody are introduced into the cells such that they are expressible by the cells or their progeny, and the recombinant cells are then administered in vivo for therapeutic effect. In a specific embodiment, stem or progenitor cells are used. Any stem and/or progenitor cells which can be isolated and maintained in vitro can potentially be used in accordance with this embodiment of the present invention (see e.g. PCT Publication WO 94/08598; Stemple and Anderson, Cell 71:973-985 (1992); Rheinwald, Meth. Cell Bio. 21A:229 (1980); and Pittelkow and Scott, Mayo Clinic Proc. 61:771 (1986)).

20 In a specific embodiment, the nucleic acid to be introduced for purposes of gene therapy comprises an inducible promoter operably linked to the coding region, such that expression of the nucleic acid is controllable by controlling the presence or absence of the appropriate inducer of transcription. Demonstration of Therapeutic or Prophylactic Activity

25 The compounds or pharmaceutical compositions of the invention are preferably tested in vitro, and then in vivo for the desired therapeutic or prophylactic activity, prior to use in humans. For example, in vitro assays to demonstrate the



therapeutic or prophylactic utility of a compound or pharmaceutical composition include, the effect of a compound on a cell line or a patient tissue sample. The effect of the compound or composition on the cell line and/or tissue sample can be determined utilizing techniques known to those of skill in the art including, but not limited to, rosette formation assays and cell lysis assays. In accordance with the invention, in vitro assays which can be used to determine whether administration of a specific compound is indicated, include in vitro cell culture assays in which a patient tissue sample is grown in culture, and exposed to or otherwise administered a compound, and the effect of such compound upon the tissue sample is observed.

10

#### Therapeutic/Prophylactic Administration and Composition

The invention provides methods of treatment, inhibition and prophylaxis by administration to a subject of an effective amount of a compound or pharmaceutical composition of the invention, preferably an antibody of the invention. In a preferred aspect, the compound is substantially purified (e.g., substantially free from substances that limit its effect or produce undesired side-effects). The subject is preferably an animal, including but not limited to animals such as cows, pigs, horses, chickens, cats, dogs, etc., and is preferably a mammal, and most preferably human.

15

Formulations and methods of administration that can be employed when the compound comprises a nucleic acid or an immunoglobulin are described above; additional appropriate formulations and routes of administration can be selected from among those described herein below.

20

Various delivery systems are known and can be used to administer a compound of the invention, e.g., encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the compound, receptor-mediated endocytosis (see, e.g., Wu and Wu, J. Biol. Chem. 262:4429-4432 (1987)),

25

construction of a nucleic acid as part of a retroviral or other vector, etc. Methods of introduction include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The compounds or compositions may be administered by any convenient route, for example by infusion  
5 or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, it may be desirable to introduce the pharmaceutical compounds or compositions of the invention into the central nervous system by any suitable route,  
10 including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary administration can also be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

In a specific embodiment, it may be desirable to administer the pharmaceutical  
15 compounds or compositions of the invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, e.g., in conjunction with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material,  
20 including membranes, such as sialastic membranes, or fibers. Preferably, when administering a protein, including an antibody, of the invention, care must be taken to use materials to which the protein does not absorb.

In another embodiment, the compound or composition can be delivered in a vesicle, in particular a liposome (see Langer, *Science* 249:1527-1533 (1990); Treat et al., in *Liposomes in the Therapy of Infectious Disease and Cancer*, Lopez-Berestein  
25

and Fidler (eds.), Liss, New York, pp. 353- 365 (1989); Lopez-Berestein, *ibid.*, pp. 317-327; see generally *ibid.*)

In yet another embodiment, the compound or composition can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer, supra; Sefton, *CRC Crit. Ref. Biomed. Eng.* 14:201 (1987); Buchwald et al., *Surgery* 88:507 (1980); Saudek et al., *N. Engl. J. Med.* 321:574 (1989)). In another embodiment, polymeric materials can be used (see *Medical Applications of Controlled Release*, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); *Controlled Drug Bioavailability, Drug Product Design and Performance*, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, J., *Macromol. Sci. Rev. Macromol. Chem.* 23:61 (1983); see also Levy et al., *Science* 228:190 (1985); During et al., *Ann. Neurol.* 25:351 (1989); Howard et al., *J. Neurosurg.* 71:105 (1989)). In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target, i.e., the brain, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, in *Medical Applications of Controlled Release*, supra, vol. 2, pp. 115-138 (1984)).

Other controlled release systems are discussed in the review by Langer (*Science* 249:1527-1533 (1990)).

In a specific embodiment where the compound of the invention is a nucleic acid encoding a protein, the nucleic acid can be administered *in vivo* to promote expression of its encoded protein, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, e.g., by use of a retroviral vector (see U.S. Patent No. 4,980,286), or by direct injection, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox- like peptide which is known to enter the nucleus (see e.g.,

Joliot et al., Proc. Natl. Acad. Sci. USA 88:1864-1868 (1991)), etc. Alternatively, a nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination.

The present invention also provides pharmaceutical compositions. Such compositions comprise a therapeutically effective amount of a compound, and a pharmaceutically acceptable carrier. In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable

pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin. Such compositions will contain a therapeutically effective amount of the compound, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation  
5 should suit the mode of administration.

In a preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the  
10 composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the  
15 composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

The compounds of the invention can be formulated as neutral or salt forms.  
20 Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

25 The amount of the compound of the invention which will be effective in the treatment, inhibition and prevention of a disease or disorder associated with aberrant

expression and/or activity of a polypeptide of the invention can be determined by standard clinical techniques. In addition, in vitro assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from in vitro or animal model test systems.

For antibodies, the dosage administered to a patient is typically 0.1 mg/kg to 100 mg/kg of the patient's body weight. Preferably, the dosage administered to a patient is between 0.1 mg/kg and 20 mg/kg of the patient's body weight, more preferably 1 mg/kg to 10 mg/kg of the patient's body weight. Generally, human antibodies have a longer half-life within the human body than antibodies from other species due to the immune response to the foreign polypeptides. Thus, lower dosages of human antibodies and less frequent administration is often possible. Further, the dosage and frequency of administration of antibodies of the invention may be reduced by enhancing uptake and tissue penetration (e.g., into the brain) of the antibodies by modifications such as, for example, lipidation.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. Diagnosis and Imaging

Labeled antibodies, and derivatives and analogs thereof, which specifically bind to a polypeptide of interest can be used for diagnostic purposes to detect,

diagnose, or monitor diseases, disorders, and/or conditions associated with the aberrant expression and/or activity of a polypeptide of the invention. The invention provides for the detection of aberrant expression of a polypeptide of interest, comprising (a) assaying the expression of the polypeptide of interest in cells or body fluid of an individual using one or more antibodies specific to the polypeptide interest and (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of aberrant expression.

The invention provides a diagnostic assay for diagnosing a disorder, comprising (a) assaying the expression of the polypeptide of interest in cells or body fluid of an individual using one or more antibodies specific to the polypeptide interest and (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of a particular disorder. With respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

Antibodies of the invention can be used to assay protein levels in a biological sample using classical immunohistological methods known to those of skill in the art (e.g., see Jalkanen, et al., J. Cell. Biol. 101:976-985 (1985); Jalkanen, et al., J. Cell Biol. 105:3087-3096 (1987)). Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody

assay labels are known in the art and include enzyme labels, such as, glucose oxidase; radioisotopes, such as iodine ( $^{125}\text{I}$ ,  $^{121}\text{I}$ ), carbon ( $^{14}\text{C}$ ), sulfur ( $^{35}\text{S}$ ), tritium ( $^3\text{H}$ ), indium ( $^{112}\text{In}$ ), and technetium ( $^{99}\text{Tc}$ ); luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin.

5           One aspect of the invention is the detection and diagnosis of a disease or disorder associated with aberrant expression of a polypeptide of interest in an animal, preferably a mammal and most preferably a human. In one embodiment, diagnosis comprises: a) administering (for example, parenterally, subcutaneously, or intraperitoneally) to a subject an effective amount of a labeled molecule which  
10 specifically binds to the polypeptide of interest; b) waiting for a time interval following the administering for permitting the labeled molecule to preferentially concentrate at sites in the subject where the polypeptide is expressed (and for unbound labeled molecule to be cleared to background level); c) determining background level; and d) detecting the labeled molecule in the subject, such that  
15 detection of labeled molecule above the background level indicates that the subject has a particular disease or disorder associated with aberrant expression of the polypeptide of interest. Background level can be determined by various methods including, comparing the amount of labeled molecule detected to a standard value previously determined for a particular system.

20           It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of  $^{99\text{mTc}}$ . The labeled antibody or antibody fragment will then preferentially  
25 accumulate at the location of cells which contain the specific protein. In vivo tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of



Radiolabeled Antibodies and Their Fragments.” (Chapter 13 in Tumor Imaging: The Radiochemical Detection of Cancer, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982).

Depending on several variables, including the type of label used and the mode  
5 of administration, the time interval following the administration for permitting the labeled molecule to preferentially concentrate at sites in the subject and for unbound labeled molecule to be cleared to background level is 6 to 48 hours or 6 to 24 hours or 6 to 12 hours. In another embodiment the time interval following administration is 5 to 20 days or 5 to 10 days.

10 In an embodiment, monitoring of the disease or disorder is carried out by repeating the method for diagnosing the disease or disease, for example, one month after initial diagnosis, six months after initial diagnosis, one year after initial diagnosis, etc.

Presence of the labeled molecule can be detected in the patient using methods  
15 known in the art for in vivo scanning. These methods depend upon the type of label used. Skilled artisans will be able to determine the appropriate method for detecting a particular label. Methods and devices that may be used in the diagnostic methods of the invention include, but are not limited to, computed tomography (CT), whole body scan such as position emission tomography (PET), magnetic resonance imaging  
20 (MRI), and sonography.

In a specific embodiment, the molecule is labeled with a radioisotope and is detected in the patient using a radiation responsive surgical instrument (Thurston et al., U.S. Patent No. 5,441,050). In another embodiment, the molecule is labeled with a fluorescent compound and is detected in the patient using a fluorescence responsive  
25 scanning instrument. In another embodiment, the molecule is labeled with a positron emitting metal and is detected in the patent using positron emission-tomography. In

yet another embodiment, the molecule is labeled with a paramagnetic label and is detected in a patient using magnetic resonance imaging (MRI).

### Kits

The present invention provides kits that can be used in the above methods. In one embodiment, a kit comprises an antibody of the invention, preferably a purified antibody, in one or more containers. In a specific embodiment, the kits of the present invention contain a substantially isolated polypeptide comprising an epitope which is specifically immunoreactive with an antibody included in the kit. Preferably, the kits of the present invention further comprise a control antibody which does not react with the polypeptide of interest. In another specific embodiment, the kits of the present invention contain a means for detecting the binding of an antibody to a polypeptide of interest (e.g., the antibody may be conjugated to a detectable substrate such as a fluorescent compound, an enzymatic substrate, a radioactive compound or a luminescent compound, or a second antibody which recognizes the first antibody may be conjugated to a detectable substrate).

In another specific embodiment of the present invention, the kit is a diagnostic kit for use in screening serum containing antibodies specific against proliferative and/or cancerous polynucleotides and polypeptides. Such a kit may include a control antibody that does not react with the polypeptide of interest. Such a kit may include a substantially isolated polypeptide antigen comprising an epitope which is specifically immunoreactive with at least one anti-polypeptide antigen antibody. Further, such a kit includes means for detecting the binding of said antibody to the antigen (e.g., the antibody may be conjugated to a fluorescent compound such as fluorescein or rhodamine which can be detected by flow cytometry). In specific embodiments, the kit may include a recombinantly produced or chemically synthesized polypeptide antigen. The polypeptide antigen of the kit may also be attached to a solid support.

In a more specific embodiment the detecting means of the above-described kit includes a solid support to which said polypeptide antigen is attached. Such a kit may also include a non-attached reporter-labeled anti-human antibody. In this embodiment, binding of the antibody to the polypeptide antigen can be detected by  
5 binding of the said reporter-labeled antibody.

In an additional embodiment, the invention includes a diagnostic kit for use in screening serum containing antigens of the polypeptide of the invention. The diagnostic kit includes a substantially isolated antibody specifically immunoreactive with polypeptide or polynucleotide antigens, and means for detecting the binding of  
10 the polynucleotide or polypeptide antigen to the antibody. In one embodiment, the antibody is attached to a solid support. In a specific embodiment, the antibody may be a monoclonal antibody. The detecting means of the kit may include a second, labeled monoclonal antibody. Alternatively, or in addition, the detecting means may include a labeled, competing antigen.

15 In one diagnostic configuration, test serum is reacted with a solid phase reagent having a surface-bound antigen obtained by the methods of the present invention. After binding with specific antigen antibody to the reagent and removing unbound serum components by washing, the reagent is reacted with reporter-labeled anti-human antibody to bind reporter to the reagent in proportion to the amount of  
20 bound anti-antigen antibody on the solid support. The reagent is again washed to remove unbound labeled antibody, and the amount of reporter associated with the reagent is determined. Typically, the reporter is an enzyme which is detected by incubating the solid phase in the presence of a suitable fluorometric, luminescent or colorimetric substrate (Sigma, St. Louis, MO).

25 The solid surface reagent in the above assay is prepared by known techniques for attaching protein material to solid support material, such as polymeric beads, dip

sticks, 96-well plate or filter material. These attachment methods generally include non-specific adsorption of the protein to the support or covalent attachment of the protein, typically through a free amine group, to a chemically reactive group on the solid support, such as an activated carboxyl, hydroxyl, or aldehyde group.

- 5 Alternatively, streptavidin coated plates can be used in conjunction with biotinylated antigen(s).

Thus, the invention provides an assay system or kit for carrying out this diagnostic method. The kit generally includes a support with surface-bound recombinant antigens, and a reporter-labeled anti-human antibody for detecting  
10 surface-bound anti-antigen antibody.

### **Fusion Proteins**

Any polypeptide of the present invention can be used to generate fusion proteins. For example, the polypeptide of the present invention, when fused to a  
15 second protein, can be used as an antigenic tag. Antibodies raised against the polypeptide of the present invention can be used to indirectly detect the second protein by binding to the polypeptide. Moreover, because secreted proteins target cellular locations based on trafficking signals, the polypeptides of the present invention can be used as targeting molecules once fused to other proteins.

20 Examples of domains that can be fused to polypeptides of the present invention include not only heterologous signal sequences, but also other heterologous functional regions. The fusion does not necessarily need to be direct, but may occur through linker sequences.

Moreover, fusion proteins may also be engineered to improve characteristics  
25 of the polypeptide of the present invention. For instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the

polypeptide to improve stability and persistence during purification from the host cell or subsequent handling and storage. Also, peptide moieties may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. The addition of peptide moieties to facilitate handling  
5 of polypeptides are familiar and routine techniques in the art.

Moreover, polypeptides of the present invention, including fragments, and specifically epitopes, can be combined with parts of the constant domain of immunoglobulins (IgA, IgE, IgG, IgM) or portions thereof (CH1, CH2, CH3, and any combination thereof, including both entire domains and portions thereof), resulting in  
10 chimeric polypeptides. These fusion proteins facilitate purification and show an increased half-life in vivo. One reported example describes chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. (EP A 394,827; Traunecker et al., Nature 331:84-86 (1988).)  
15 Fusion proteins having disulfide-linked dimeric structures (due to the IgG) can also be more efficient in binding and neutralizing other molecules, than the monomeric secreted protein or protein fragment alone. (Fountoulakis et al., J. Biochem. 270:3958-3964 (1995).)

Similarly, EP-A-O 464 533 (Canadian counterpart 2045869) discloses fusion  
20 proteins comprising various portions of constant region of immunoglobulin molecules together with another human protein or part thereof. In many cases, the Fc part in a fusion protein is beneficial in therapy and diagnosis, and thus can result in, for example, improved pharmacokinetic properties. (EP-A 0232 262.) Alternatively, deleting the Fc part after the fusion protein has been expressed, detected, and purified,  
25 would be desired. For example, the Fc portion may hinder therapy and diagnosis if the fusion protein is used as an antigen for immunizations. In drug discovery, for

example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. (See, D. Bennett et al., J. Molecular Recognition 8:52-58 (1995); K. Johanson et al., J. Biol. Chem. 270:9459-9471 (1995).)

- 5           Moreover, the polypeptides of the present invention can be fused to marker sequences, such as a peptide which facilitates purification of the fused polypeptide. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available.
- 10       As described in Gentz et al., Proc. Natl. Acad. Sci. USA 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Another peptide tag useful for purification, the "HA" tag, corresponds to an epitope derived from the influenza hemagglutinin protein. (Wilson et al., Cell 37:767 (1984).)
- 15           Thus, any of these above fusions can be engineered using the polynucleotides or the polypeptides of the present invention.

#### **Vectors, Host Cells, and Protein Production**

- 20       The present invention also relates to vectors containing the polynucleotide of the present invention, host cells, and the production of polypeptides by recombinant techniques. The vector may be, for example, a phage, plasmid, viral, or retroviral vector. Retroviral vectors may be replication competent or replication defective. In the latter case, viral propagation generally will occur only in complementing host cells.

- 25       The polynucleotides may be joined to a vector containing a selectable marker for propagation in a host. Generally, a plasmid vector is introduced in a precipitate,

such as a calcium phosphate precipitate, or in a complex with a charged lipid. If the vector is a virus, it may be packaged in vitro using an appropriate packaging cell line and then transduced into host cells.

The polynucleotide insert should be operatively linked to an appropriate  
5 promoter, such as the phage lambda PL promoter, the *E. coli* lac, trp, phoA and tac promoters, the SV40 early and late promoters and promoters of retroviral LTRs, to name a few. Other suitable promoters will be known to the skilled artisan. The expression constructs will further contain sites for transcription initiation, termination, and, in the transcribed region, a ribosome binding site for translation. The coding  
10 portion of the transcripts expressed by the constructs will preferably include a translation initiating codon at the beginning and a termination codon (UAA, UGA or UAG) appropriately positioned at the end of the polypeptide to be translated.

As indicated, the expression vectors will preferably include at least one selectable marker. Such markers include dihydrofolate reductase, G418 or neomycin  
15 resistance for eukaryotic cell culture and tetracycline, kanamycin or ampicillin resistance genes for culturing in *E. coli* and other bacteria. Representative examples of appropriate hosts include, but are not limited to, bacterial cells, such as *E. coli*, *Streptomyces* and *Salmonella typhimurium* cells; fungal cells, such as yeast cells (e.g., *Saccharomyces cerevisiae* or *Pichia pastoris* (ATCC Accession No. 201178));  
20 insect cells such as *Drosophila* S2 and *Spodoptera Sf9* cells; animal cells such as CHO, COS, 293, and Bowes melanoma cells; and plant cells. Appropriate culture mediums and conditions for the above-described host cells are known in the art.

Among vectors preferred for use in bacteria include pQE70, pQE60 and pQE-  
9, available from QIAGEN, Inc.; pBluescript vectors, Phagescript vectors, pNH8A,  
25 pNH16a, pNH18A, pNH46A, available from Stratagene Cloning Systems, Inc.; and ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 available from Pharmacia Biotech,

Inc. Among preferred eukaryotic vectors are pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; and pSVK3, pBPV, pMSG and pSVL available from Pharmacia. Preferred expression vectors for use in yeast systems include, but are not limited to pYES2, pYD1, pTEF1/Zeo, pYES2/GS, pPICZ,pGAPZ, pGAPZalph,  
5 pPIC9, pPIC3.5, pHIL-D2, pHIL-S1, pPIC3.5K, pPIC9K, and PAO815 (all available from Invitrogen, Carlsbad, CA). Other suitable vectors will be readily apparent to the skilled artisan.

Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated  
10 transfection, electroporation, transduction, infection, or other methods. Such methods are described in many standard laboratory manuals, such as Davis et al., Basic Methods In Molecular Biology (1986). It is specifically contemplated that the polypeptides of the present invention may in fact be expressed by a host cell lacking a recombinant vector.

15 A polypeptide of this invention can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most  
20 preferably, high performance liquid chromatography ("HPLC") is employed for purification.

Polypeptides of the present invention, and preferably the secreted form, can also be recovered from: products purified from natural sources, including bodily fluids, tissues and cells, whether directly isolated or cultured; products of chemical  
25 synthetic procedures; and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant,



insect, and mammalian cells. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. In addition, polypeptides of the invention may also include an initial modified methionine residue, in some cases as a result of host-mediated processes. Thus, it is well known in the art that the N-terminal methionine encoded by the translation initiation codon generally is removed with high efficiency from any protein after translation in all eukaryotic cells. While the N-terminal methionine on most proteins also is efficiently removed in most prokaryotes, for some proteins, this prokaryotic removal process is inefficient, depending on the nature of the amino acid to which the N-terminal methionine is covalently linked.

In one embodiment, the yeast *Pichia pastoris* is used to express the polypeptide of the present invention in a eukaryotic system. *Pichia pastoris* is a methylotrophic yeast which can metabolize methanol as its sole carbon source. A main step in the methanol metabolism pathway is the oxidation of methanol to formaldehyde using O<sub>2</sub>. This reaction is catalyzed by the enzyme alcohol oxidase. In order to metabolize methanol as its sole carbon source, *Pichia pastoris* must generate high levels of alcohol oxidase due, in part, to the relatively low affinity of alcohol oxidase for O<sub>2</sub>. Consequently, in a growth medium depending on methanol as a main carbon source, the promoter region of one of the two alcohol oxidase genes (*AOX1*) is highly active. In the presence of methanol, alcohol oxidase produced from the *AOX1* gene comprises up to approximately 30% of the total soluble protein in *Pichia pastoris*. See, Ellis, S.B., *et al.*, *Mol. Cell. Biol.* 5:1111-21 (1985); Koutz, P.J., *et al.*, *Yeast* 5:167-77 (1989); Tschopp, J.F., *et al.*, *Nucl. Acids Res.* 15:3859-76 (1987). Thus, a heterologous coding sequence, such as, for example, a polynucleotide of the present invention, under the transcriptional regulation of all or part of the *AOX1*

regulatory sequence is expressed at exceptionally high levels in *Pichia* yeast grown in the presence of methanol.

In one example, the plasmid vector pPIC9K is used to express DNA encoding a polypeptide of the invention, as set forth herein, in a *Pichea* yeast system essentially as described in "*Pichia* Protocols: Methods in Molecular Biology," D.R. Higgins and J. Cregg, eds. The Humana Press, Totowa, NJ, 1998. This expression vector allows expression and secretion of a protein of the invention by virtue of the strong *AOX1* promoter linked to the *Pichia pastoris* alkaline phosphatase (PHO) secretory signal peptide (i.e., leader) located upstream of a multiple cloning site.

Many other yeast vectors could be used in place of pPIC9K, such as, pYES2, pYD1, pTEF1/Zeo, pYES2/GS, pPICZ, pGAPZ, pGAPZalpha, pPIC9, pPIC3.5, pHIL-D2, pHIL-S1, pPIC3.5K, and PAO815, as one skilled in the art would readily appreciate, as long as the proposed expression construct provides appropriately located signals for transcription, translation, secretion (if desired), and the like, including an in-frame AUG as required.

In another embodiment, high-level expression of a heterologous coding sequence, such as, for example, a polynucleotide of the present invention, may be achieved by cloning the heterologous polynucleotide of the invention into an expression vector such as, for example, pGAPZ or pGAPZalpha, and growing the yeast culture in the absence of methanol.

In addition to encompassing host cells containing the vector constructs discussed herein, the invention also encompasses primary, secondary, and immortalized host cells of vertebrate origin, particularly mammalian origin, that have been engineered to delete or replace endogenous genetic material (e.g., coding sequence), and/or to include genetic material (e.g., heterologous polynucleotide sequences) that is operably associated with the polynucleotides of the invention, and

which activates, alters, and/or amplifies endogenous polynucleotides. For example, techniques known in the art may be used to operably associate heterologous control regions (e.g., promoter and/or enhancer) and endogenous polynucleotide sequences via homologous recombination, resulting in the formation of a new transcription unit (see, e.g., U.S. Patent No. 5,641,670, issued June 24, 1997; U.S. Patent No. 5,733,761, issued March 31, 1998; International Publication No. WO 96/29411, published September 26, 1996; International Publication No. WO 94/12650, published August 4, 1994; Koller et al., Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); and Zijlstra et al., Nature 342:435-438 (1989), the disclosures of each of which are incorporated by reference in their entireties).

In addition, polypeptides of the invention can be chemically synthesized using techniques known in the art (e.g., see Creighton, 1983, Proteins: Structures and Molecular Principles, W.H. Freeman & Co., N.Y., and Hunkapiller et al., *Nature*, 310:105-111 (1984)). For example, a polypeptide corresponding to a fragment of a polypeptide sequence of the invention can be synthesized by use of a peptide synthesizer. Furthermore, if desired, nonclassical amino acids or chemical amino acid analogs can be introduced as a substitution or addition into the polypeptide sequence. Non-classical amino acids include, but are not limited to, to the D-isomers of the common amino acids, 2,4-diaminobutyric acid,  $\alpha$ -amino isobutyric acid, 4-aminobutyric acid, Abu, 2-amino butyric acid, g-Abu, e-Ahx, 6-amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, homocitrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, b-alanine, fluoro-amino acids, designer amino acids such as b-methyl amino acids, Ca-methyl amino acids, Na-methyl amino acids, and amino acid analogs in general. Furthermore, the amino acid can be D (dextrorotary) or L (levorotary).

The invention encompasses polypeptides which are differentially modified during or after translation, *e.g.*, by glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. Any of numerous

5 chemical modifications may be carried out by known techniques, including but not limited, to specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease, NaBH<sub>4</sub>; acetylation, formylation, oxidation, reduction; metabolic synthesis in the presence of tunicamycin; etc.

Additional post-translational modifications encompassed by the invention

10 include, for example, *e.g.*, N-linked or O-linked carbohydrate chains, processing of N-terminal or C-terminal ends), attachment of chemical moieties to the amino acid backbone, chemical modifications of N-linked or O-linked carbohydrate chains, and addition or deletion of an N-terminal methionine residue as a result of procaryotic host cell expression. The polypeptides may also be modified with a detectable label,

15 such as an enzymatic, fluorescent, isotopic or affinity label to allow for detection and isolation of the protein.

Also provided by the invention are chemically modified derivatives of the polypeptides of the invention which may provide additional advantages such as increased solubility, stability and circulating time of the polypeptide, or decreased

20 immunogenicity (see U.S. Patent NO: 4,179,337). The chemical moieties for derivitization may be selected from water soluble polymers such as polyethylene glycol, ethylene glycol/propylene glycol copolymers, carboxymethylcellulose, dextran, polyvinyl alcohol and the like. The polypeptides may be modified at random positions within the molecule, or at predetermined positions within the molecule and

25 may include one, two, three or more attached chemical moieties.

The polymer may be of any molecular weight, and may be branched or unbranched. For polyethylene glycol, the preferred molecular weight is between about 1 kDa and about 100 kDa (the term "about" indicating that in preparations of polyethylene glycol, some molecules will weigh more, some less, than the stated  
5 molecular weight) for ease in handling and manufacturing. Other sizes may be used, depending on the desired therapeutic profile (e.g., the duration of sustained release desired, the effects, if any on biological activity, the ease in handling, the degree or lack of antigenicity and other known effects of the polyethylene glycol to a therapeutic protein or analog).

10 The polyethylene glycol molecules (or other chemical moieties) should be attached to the protein with consideration of effects on functional or antigenic domains of the protein. There are a number of attachment methods available to those skilled in the art, e.g., EP 0 401 384, herein incorporated by reference (coupling PEG to G-CSF), see also Malik et al., Exp. Hematol. 20:1028-1035 (1992) (reporting  
15 pegylation of GM-CSF using tresyl chloride). For example, polyethylene glycol may be covalently bound through amino acid residues via a reactive group, such as, a free amino or carboxyl group. Reactive groups are those to which an activated polyethylene glycol molecule may be bound. The amino acid residues having a free amino group may include lysine residues and the N-terminal amino acid residues;  
20 those having a free carboxyl group may include aspartic acid residues glutamic acid residues and the C-terminal amino acid residue. Sulfhydryl groups may also be used as a reactive group for attaching the polyethylene glycol molecules. Preferred for therapeutic purposes is attachment at an amino group, such as attachment at the N-terminus or lysine group.

25 One may specifically desire proteins chemically modified at the N-terminus. Using polyethylene glycol as an illustration of the present composition, one may

select from a variety of polyethylene glycol molecules (by molecular weight, branching, etc.), the proportion of polyethylene glycol molecules to protein (polypeptide) molecules in the reaction mix, the type of pegylation reaction to be performed, and the method of obtaining the selected N-terminally pegylated protein.

- 5 The method of obtaining the N-terminally pegylated preparation (i.e., separating this moiety from other monopegylated moieties if necessary) may be by purification of the N-terminally pegylated material from a population of pegylated protein molecules. Selective proteins chemically modified at the N-terminus modification may be accomplished by reductive alkylation which exploits differential reactivity of different
- 10 types of primary amino groups (lysine versus the N-terminal) available for derivatization in a particular protein. Under the appropriate reaction conditions, substantially selective derivatization of the protein at the N-terminus with a carbonyl group containing polymer is achieved.

- The polypeptides of the invention may be in monomers or multimers (i.e.,
- 15 dimers, trimers, tetramers and higher multimers). Accordingly, the present invention relates to monomers and multimers of the polypeptides of the invention, their preparation, and compositions (preferably, *Therapeutics*) containing them. In specific embodiments, the polypeptides of the invention are monomers, dimers, trimers or tetramers. In additional embodiments, the multimers of the invention are at least
- 20 dimers, at least trimers, or at least tetramers.

- Multimers encompassed by the invention may be homomers or heteromers. As used herein, the term homomer, refers to a multimer containing only polypeptides corresponding to the amino acid sequence of SEQ ID NO:Y or encoded by the cDNA contained in a deposited clone (including fragments, variants, splice variants, and
- 25 fusion proteins, corresponding to these polypeptides as described herein). These homomers may contain polypeptides having identical or different amino acid

sequences. In a specific embodiment, a homomer of the invention is a multimer containing only polypeptides having an identical amino acid sequence. In another specific embodiment, a homomer of the invention is a multimer containing polypeptides having different amino acid sequences. In specific embodiments, the multimer of the invention is a homodimer (*e.g.*, containing polypeptides having identical or different amino acid sequences) or a homotrimer (*e.g.*, containing polypeptides having identical and/or different amino acid sequences). In additional embodiments, the homomeric multimer of the invention is at least a homodimer, at least a homotrimer, or at least a homotetramer.

10 As used herein, the term heteromer refers to a multimer containing one or more heterologous polypeptides (*i.e.*, polypeptides of different proteins) in addition to the polypeptides of the invention. In a specific embodiment, the multimer of the invention is a heterodimer, a heterotrimer, or a heterotetramer. In additional embodiments, the heteromeric multimer of the invention is at least a heterodimer, at least a heterotrimer, or at least a heterotetramer.

Multimers of the invention may be the result of hydrophobic, hydrophilic, ionic and/or covalent associations and/or may be indirectly linked, by for example, liposome formation. Thus, in one embodiment, multimers of the invention, such as, for example, homodimers or homotrimers, are formed when polypeptides of the invention contact one another in solution. In another embodiment, heteromultimers of the invention, such as, for example, heterotrimers or heterotetramers, are formed when polypeptides of the invention contact antibodies to the polypeptides of the invention (including antibodies to the heterologous polypeptide sequence in a fusion protein of the invention) in solution. In other embodiments, multimers of the invention are formed by covalent associations with and/or between the polypeptides of the invention. Such covalent associations may involve one or more amino acid

residues contained in the polypeptide sequence ( e.g., that recited in the sequence listing, or contained in the polypeptide encoded by a deposited clone). In one instance, the covalent associations are cross-linking between cysteine residues located within the polypeptide sequences which interact in the native (i.e., naturally occurring) polypeptide. In another instance, the covalent associations are the consequence of chemical or recombinant manipulation. Alternatively, such covalent associations may involve one or more amino acid residues contained in the heterologous polypeptide sequence in a fusion protein of the invention.

In one example, covalent associations are between the heterologous sequence contained in a fusion protein of the invention (see, e.g., US Patent Number 5,478,925). In a specific example, the covalent associations are between the heterologous sequence contained in an Fc fusion protein of the invention (as described herein). In another specific example, covalent associations of fusion proteins of the invention are between heterologous polypeptide sequence from another protein that is capable of forming covalently associated multimers, such as for example, osteopontin (see, e.g., International Publication NO: WO 98/49305, the contents of which are herein incorporated by reference in its entirety). In another embodiment, two or more polypeptides of the invention are joined through peptide linkers. Examples include those peptide linkers described in U.S. Pat. No. 5,073,627 (hereby incorporated by reference). Proteins comprising multiple polypeptides of the invention separated by peptide linkers may be produced using conventional recombinant DNA technology.

Another method for preparing multimer polypeptides of the invention involves use of polypeptides of the invention fused to a leucine zipper or isoleucine zipper polypeptide sequence. Leucine zipper and isoleucine zipper domains are polypeptides that promote multimerization of the proteins in which they are found. Leucine



zippers were originally identified in several DNA-binding proteins (Landschulz et al., Science 240:1759, (1988)), and have since been found in a variety of different proteins. Among the known leucine zippers are naturally occurring peptides and derivatives thereof that dimerize or trimerize. Examples of leucine zipper domains  
5 suitable for producing soluble multimeric proteins of the invention are those described in PCT application WO 94/10308, hereby incorporated by reference. Recombinant fusion proteins comprising a polypeptide of the invention fused to a polypeptide sequence that dimerizes or trimerizes in solution are expressed in suitable host cells, and the resulting soluble multimeric fusion protein is recovered from the culture  
10 supernatant using techniques known in the art.

Trimeric polypeptides of the invention may offer the advantage of enhanced biological activity. Preferred leucine zipper moieties and isoleucine moieties are those that preferentially form trimers. One example is a leucine zipper derived from lung surfactant protein D (SPD), as described in Hoppe et al. (FEBS Letters 344:191,  
15 (1994)) and in U.S. patent application Ser. No. 08/446,922, hereby incorporated by reference. Other peptides derived from naturally occurring trimeric proteins may be employed in preparing trimeric polypeptides of the invention.

In another example, proteins of the invention are associated by interactions between Flag® polypeptide sequence contained in fusion proteins of the invention  
20 containing Flag® polypeptide sequence. In a further embodiment, associations proteins of the invention are associated by interactions between heterologous polypeptide sequence contained in Flag® fusion proteins of the invention and anti-Flag® antibody.

The multimers of the invention may be generated using chemical techniques  
25 known in the art. For example, polypeptides desired to be contained in the multimers of the invention may be chemically cross-linked using linker molecules and linker

molecule length optimization techniques known in the art (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety).

Additionally, multimers of the invention may be generated using techniques known in the art to form one or more inter-molecule cross-links between the cysteine residues

5 located within the sequence of the polypeptides desired to be contained in the multimer (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Further, polypeptides of the invention may be routinely modified by the addition of cysteine or biotin to the C terminus or N-terminus of the polypeptide and techniques known in the art may be applied to generate multimers  
10 containing one or more of these modified polypeptides (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Additionally, techniques known in the art may be applied to generate liposomes containing the polypeptide components desired to be contained in the multimer of the invention (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its  
15 entirety).

Alternatively, multimers of the invention may be generated using genetic engineering techniques known in the art. In one embodiment, polypeptides contained in multimers of the invention are produced recombinantly using fusion protein technology described herein or otherwise known in the art (see, e.g., US Patent  
20 Number 5,478,925, which is herein incorporated by reference in its entirety). In a specific embodiment, polynucleotides coding for a homodimer of the invention are generated by ligating a polynucleotide sequence encoding a polypeptide of the invention to a sequence encoding a linker polypeptide and then further to a synthetic polynucleotide encoding the translated product of the polypeptide in the reverse  
25 orientation from the original C-terminus to the N-terminus (lacking the leader sequence) (see, e.g., US Patent Number 5,478,925, which is herein incorporated by

reference in its entirety). In another embodiment, recombinant techniques described herein or otherwise known in the art are applied to generate recombinant polypeptides of the invention which contain a transmembrane domain (or hydrophobic or signal peptide) and which can be incorporated by membrane reconstitution techniques into liposomes (see, e.g., US Patent Number 5,478,925, which is herein incorporated by  
5 reference in its entirety).

### **Uses of the Polynucleotides**

Each of the polynucleotides identified herein can be used in numerous ways as reagents. The following description should be considered exemplary and utilizes  
10 known techniques.

The polynucleotides of the present invention are useful for chromosome identification. There exists an ongoing need to identify new chromosome markers, since few chromosome marking reagents, based on actual sequence data (repeat polymorphisms), are presently available. Each polynucleotide of the present  
15 invention can be used as a chromosome marker.

Briefly, sequences can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp) from the sequences shown in SEQ ID NO:X. Primers can be selected using computer analysis so that primers do not span more than one predicted  
20 exon in the genomic DNA. These primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the SEQ ID NO:X will yield an amplified fragment.

Similarly, somatic hybrids provide a rapid method of PCR mapping the  
25 polynucleotides to particular chromosomes. Three or more clones can be assigned per day using a single thermal cycler. Moreover, sublocalization of the polynucleotides

can be achieved with panels of specific chromosome fragments. Other gene mapping strategies that can be used include in situ hybridization, prescreening with labeled flow-sorted chromosomes, and preselection by hybridization to construct chromosome specific-cDNA libraries.

5           Precise chromosomal location of the polynucleotides can also be achieved using fluorescence in situ hybridization (FISH) of a metaphase chromosomal spread. This technique uses polynucleotides as short as 500 or 600 bases; however, polynucleotides 2,000-4,000 bp are preferred. For a review of this technique, see Verma et al., "Human Chromosomes: a Manual of Basic Techniques," Pergamon  
10   Press, New York (1988).

For chromosome mapping, the polynucleotides can be used individually (to mark a single chromosome or a single site on that chromosome) or in panels (for marking multiple sites and/or multiple chromosomes). Preferred polynucleotides correspond to the noncoding regions of the cDNAs because the coding sequences are  
15   more likely conserved within gene families, thus increasing the chance of cross hybridization during chromosomal mapping.

Once a polynucleotide has been mapped to a precise chromosomal location, the physical position of the polynucleotide can be used in linkage analysis. Linkage analysis establishes coinheritance between a chromosomal location and presentation  
20   of a particular disease. (Disease mapping data are found, for example, in V. McKusick, Mendelian Inheritance in Man (available on line through Johns Hopkins University Welch Medical Library) .) Assuming 1 megabase mapping resolution and one gene per 20 kb, a cDNA precisely localized to a chromosomal region associated with the disease could be one of 50-500 potential causative genes.

25           Thus, once coinheritance is established, differences in the polynucleotide and the corresponding gene between affected and unaffected individuals can be examined.

First, visible structural alterations in the chromosomes, such as deletions or translocations, are examined in chromosome spreads or by PCR. If no structural alterations exist, the presence of point mutations are ascertained. Mutations observed in some or all affected individuals, but not in normal individuals, indicates that the mutation may cause the disease. However, complete sequencing of the polypeptide and the corresponding gene from several normal individuals is required to distinguish the mutation from a polymorphism. If a new polymorphism is identified, this polymorphic polypeptide can be used for further linkage analysis.

Furthermore, increased or decreased expression of the gene in affected individuals as compared to unaffected individuals can be assessed using polynucleotides of the present invention. Any of these alterations (altered expression, chromosomal rearrangement, or mutation) can be used as a diagnostic or prognostic marker.

Thus, the invention also provides a diagnostic method useful during diagnosis of a disorder, involving measuring the expression level of polynucleotides of the present invention in cells or body fluid from an individual and comparing the measured gene expression level with a standard level of polynucleotide expression level, whereby an increase or decrease in the gene expression level compared to the standard is indicative of a disorder.

In still another embodiment, the invention includes a kit for analyzing samples for the presence of proliferative and/or cancerous polynucleotides derived from a test subject. In a general embodiment, the kit includes at least one polynucleotide probe containing a nucleotide sequence that will specifically hybridize with a polynucleotide of the present invention and a suitable container. In a specific embodiment, the kit includes two polynucleotide probes defining an internal region of the polynucleotide of the present invention, where each probe has one strand

containing a 31'mer-end internal to the region. In a further embodiment, the probes may be useful as primers for polymerase chain reaction amplification.

Where a diagnosis of a disorder, has already been made according to conventional methods, the present invention is useful as a prognostic indicator, whereby patients exhibiting enhanced or depressed polynucleotide of the present invention expression will experience a worse clinical outcome relative to patients expressing the gene at a level nearer the standard level.

By "measuring the expression level of polynucleotide of the present invention" is intended qualitatively or quantitatively measuring or estimating the level of the polypeptide of the present invention or the level of the mRNA encoding the polypeptide in a first biological sample either directly (e.g., by determining or estimating absolute protein level or mRNA level) or relatively (e.g., by comparing to the polypeptide level or mRNA level in a second biological sample). Preferably, the polypeptide level or mRNA level in the first biological sample is measured or estimated and compared to a standard polypeptide level or mRNA level, the standard being taken from a second biological sample obtained from an individual not having the disorder or being determined by averaging levels from a population of individuals not having a disorder. As will be appreciated in the art, once a standard polypeptide level or mRNA level is known, it can be used repeatedly as a standard for comparison.

By "biological sample" is intended any biological sample obtained from an individual, body fluid, cell line, tissue culture, or other source which contains the polypeptide of the present invention or mRNA. As indicated, biological samples include body fluids (such as semen, lymph, sera, plasma, urine, synovial fluid and spinal fluid) which contain the polypeptide of the present invention, and other tissue sources found to express the polypeptide of the present invention. Methods for

obtaining tissue biopsies and body fluids from mammals are well known in the art. Where the biological sample is to include mRNA, a tissue biopsy is the preferred source.

The method(s) provided above may preferably be applied in a diagnostic  
5 method and/or kits in which polynucleotides and/or polypeptides are attached to a solid support. In one exemplary method, the support may be a "gene chip" or a "biological chip" as described in US Patents 5,837,832, 5,874,219, and 5,856,174. Further, such a gene chip with polynucleotides of the present invention attached may be used to identify polymorphisms between the polynucleotide sequences, with  
10 polynucleotides isolated from a test subject. The knowledge of such polymorphisms (i.e. their location, as well as, their existence) would be beneficial in identifying disease loci for many disorders, including cancerous diseases and conditions. Such a method is described in US Patents 5,858,659 and 5,856,104. The US Patents referenced supra are hereby incorporated by reference in their entirety herein.

15 The present invention encompasses polynucleotides of the present invention that are chemically synthesized, or reproduced as peptide nucleic acids (PNA), or according to other methods known in the art. The use of PNAs would serve as the preferred form if the polynucleotides are incorporated onto a solid support, or gene chip. For the purposes of the present invention, a peptide nucleic acid (PNA) is a  
20 polyamide type of DNA analog and the monomeric units for adenine, guanine, thymine and cytosine are available commercially (Perceptive Biosystems). Certain components of DNA, such as phosphorus, phosphorus oxides, or deoxyribose derivatives, are not present in PNAs. As disclosed by P. E. Nielsen, M. Egholm, R. H. Berg and O. Buchardt, Science 254, 1497 (1991); and M. Egholm, O. Buchardt,  
25 L.Christensen, C. Behrens, S. M. Freier, D. A. Driver, R. H. Berg, S. K. Kim, B. Norden, and P. E. Nielsen, Nature 365, 666 (1993), PNAs bind specifically and

tightly to complementary DNA strands and are not degraded by nucleases. In fact, PNA binds more strongly to DNA than DNA itself does. This is probably because there is no electrostatic repulsion between the two strands, and also the polyamide backbone is more flexible. Because of this, PNA/DNA duplexes bind under a wider  
5 range of stringency conditions than DNA/DNA duplexes, making it easier to perform multiplex hybridization. Smaller probes can be used than with DNA due to the strong binding. In addition, it is more likely that single base mismatches can be determined with PNA/DNA hybridization because a single mismatch in a PNA/DNA 15-mer  
10 lowers the melting point ( $T_{sub.m}$ ) by 8°-20° C, vs. 4°-16° C for the DNA/DNA 15-mer duplex. Also, the absence of charge groups in PNA means that hybridization can be done at low ionic strengths and reduce possible interference by salt during the analysis.

The present invention is useful for detecting cancer in mammals. In particular the invention is useful during diagnosis of pathological cell proliferative neoplasias  
15 which include, but are not limited to: acute myelogenous leukemias including acute monocytic leukemia, acute myeloblastic leukemia, acute promyelocytic leukemia, acute myelomonocytic leukemia, acute erythroleukemia, acute megakaryocytic leukemia, and acute undifferentiated leukemia, etc.; and chronic myelogenous leukemias including chronic myelomonocytic leukemia, chronic granulocytic  
20 leukemia, etc. Preferred mammals include monkeys, apes, cats, dogs, cows, pigs, horses, rabbits and humans. Particularly preferred are humans.

Pathological cell proliferative diseases, disorders, and/or conditions are often associated with inappropriate activation of proto-oncogenes. (Gelman, E. P. et al., "The Etiology of Acute Leukemia: Molecular Genetics and Viral Oncology," in  
25 Neoplastic Diseases of the Blood, Vol 1., Wiernik, P. H. et al. eds., 161-182 (1985)). Neoplasias are now believed to result from the qualitative alteration of a normal



cellular gene product, or from the quantitative modification of gene expression by insertion into the chromosome of a viral sequence, by chromosomal translocation of a gene to a more actively transcribed region, or by some other mechanism. (Germann et al., supra) It is likely that mutated or altered expression of specific genes is involved in the pathogenesis of some leukemias, among other tissues and cell types. (Germann et al., supra) Indeed, the human counterparts of the oncogenes involved in some animal neoplasias have been amplified or translocated in some cases of human leukemia and carcinoma. (Germann et al., supra)

For example, c-myc expression is highly amplified in the non-lymphocytic leukemia cell line HL-60. When HL-60 cells are chemically induced to stop proliferation, the level of c-myc is found to be downregulated. (International Publication Number WO 91/15580) However, it has been shown that exposure of HL-60 cells to a DNA construct that is complementary to the 5' end of c-myc or c-myb blocks translation of the corresponding mRNAs which downregulates expression of the c-myc or c-myb proteins and causes arrest of cell proliferation and differentiation of the treated cells. (International Publication Number WO 91/15580; Wickstrom et al., Proc. Natl. Acad. Sci. 85:1028 (1988); Anfossi et al., Proc. Natl. Acad. Sci. 86:3379 (1989)). However, the skilled artisan would appreciate the present invention's usefulness would not be limited to treatment of proliferative diseases, disorders, and/or conditions of hematopoietic cells and tissues, in light of the numerous cells and cell types of varying origins which are known to exhibit proliferative phenotypes.

In addition to the foregoing, a polynucleotide can be used to control gene expression through triple helix formation or antisense DNA or RNA. Antisense techniques are discussed, for example, in Okano, J. Neurochem. 56: 560 (1991); "Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Triple helix formation is discussed in, for instance Lee et al.,

Nucleic Acids Research 6: 3073 (1979); Cooney et al., Science 241: 456 (1988); and Dervan et al., Science 251: 1360 (1991). Both methods rely on binding of the polynucleotide to a complementary DNA or RNA. For these techniques, preferred polynucleotides are usually oligonucleotides 20 to 40 bases in length and  
5 complementary to either the region of the gene involved in transcription (triple helix - see Lee et al., Nucl. Acids Res. 6:3073 (1979); Cooney et al., Science 241:456 (1988); and Dervan et al., Science 251:1360 (1991) ) or to the mRNA itself (antisense - Okano, J. Neurochem. 56:560 (1991); Oligodeoxy-nucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988).) Triple helix  
10 formation optimally results in a shut-off of RNA transcription from DNA, while antisense RNA hybridization blocks translation of an mRNA molecule into polypeptide. Both techniques are effective in model systems, and the information disclosed herein can be used to design antisense or triple helix polynucleotides in an effort to treat or prevent disease.

15 Polynucleotides of the present invention are also useful in gene therapy. One goal of gene therapy is to insert a normal gene into an organism having a defective gene, in an effort to correct the genetic defect. The polynucleotides disclosed in the present invention offer a means of targeting such genetic defects in a highly accurate manner. Another goal is to insert a new gene that was not present in the host genome,  
20 thereby producing a new trait in the host cell.

The polynucleotides are also useful for identifying individuals from minute biological samples. The United States military, for example, is considering the use of restriction fragment length polymorphism (RFLP) for identification of its personnel. In this technique, an individual's genomic DNA is digested with one or more  
25 restriction enzymes, and probed on a Southern blot to yield unique bands for identifying personnel. This method does not suffer from the current limitations of

"Dog Tags" which can be lost, switched, or stolen, making positive identification difficult. The polynucleotides of the present invention can be used as additional DNA markers for RFLP.

The polynucleotides of the present invention can also be used as an alternative to RFLP, by determining the actual base-by-base DNA sequence of selected portions of an individual's genome. These sequences can be used to prepare PCR primers for amplifying and isolating such selected DNA, which can then be sequenced. Using this technique, individuals can be identified because each individual will have a unique set of DNA sequences. Once an unique ID database is established for an individual, positive identification of that individual, living or dead, can be made from extremely small tissue samples.

Forensic biology also benefits from using DNA-based identification techniques as disclosed herein. DNA sequences taken from very small biological samples such as tissues, e.g., hair or skin, or body fluids, e.g., blood, saliva, semen, synovial fluid, amniotic fluid, breast milk, lymph, pulmonary sputum or surfactant, urine, fecal matter, etc., can be amplified using PCR. In one prior art technique, gene sequences amplified from polymorphic loci, such as DQa class II HLA gene, are used in forensic biology to identify individuals. (Erich, H., PCR Technology, Freeman and Co. (1992).) Once these specific polymorphic loci are amplified, they are digested with one or more restriction enzymes, yielding an identifying set of bands on a Southern blot probed with DNA corresponding to the DQa class II HLA gene. Similarly, polynucleotides of the present invention can be used as polymorphic markers for forensic purposes.

There is also a need for reagents capable of identifying the source of a particular tissue. Such need arises, for example, in forensics when presented with tissue of unknown origin. Appropriate reagents can comprise, for example, DNA

probes or primers specific to particular tissue prepared from the sequences of the present invention. Panels of such reagents can identify tissue by species and/or by organ type. In a similar fashion, these reagents can be used to screen tissue cultures for contamination.

- 5           In the very least, the polynucleotides of the present invention can be used as molecular weight markers on Southern gels, as diagnostic probes for the presence of a specific mRNA in a particular cell type, as a probe to "subtract-out" known sequences in the process of discovering novel polynucleotides, for selecting and making oligomers for attachment to a "gene chip" or other support, to raise anti-DNA  
10   antibodies using DNA immunization techniques, and as an antigen to elicit an immune response.

#### **Uses of the Polypeptides**

- Each of the polypeptides identified herein can be used in numerous ways. The  
15   following description should be considered exemplary and utilizes known techniques.

- A polypeptide of the present invention can be used to assay protein levels in a biological sample using antibody-based techniques. For example, protein expression in tissues can be studied with classical immunohistological methods. (Jalkanen, M., et al., J. Cell. Biol. 101:976-985 (1985); Jalkanen, M., et al., J. Cell . Biol. 105:3087-  
20   3096 (1987).) Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase, and radioisotopes, such as iodine (125I, 121I), carbon (14C), sulfur (35S), tritium (3H), indium (112In), and  
25   technetium (99mTc), and fluorescent labels, such as fluorescein and rhodamine, and biotin.

In addition to assaying secreted protein levels in a biological sample, proteins can also be detected in vivo by imaging. Antibody labels or markers for in vivo imaging of protein include those detectable by X-radiography, NMR or ESR. For X-radiography, suitable labels include radioisotopes such as barium or cesium, which  
5 emit detectable radiation but are not overtly harmful to the subject. Suitable markers for NMR and ESR include those with a detectable characteristic spin, such as deuterium, which may be incorporated into the antibody by labeling of nutrients for the relevant hybridoma.

A protein-specific antibody or antibody fragment which has been labeled with  
10 an appropriate detectable imaging moiety, such as a radioisotope (for example,  $^{131}\text{I}$ ,  $^{112}\text{In}$ ,  $^{99\text{m}}\text{Tc}$ ), a radio-opaque substance, or a material detectable by nuclear magnetic resonance, is introduced (for example, parenterally, subcutaneously, or intraperitoneally) into the mammal. It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety  
15 needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of  $^{99\text{m}}\text{Tc}$ . The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells which contain the specific protein. In vivo tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics  
20 of Radiolabeled Antibodies and Their Fragments." (Chapter 13 in Tumor Imaging: The Radiochemical Detection of Cancer, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982).)

Thus, the invention provides a diagnostic method of a disorder, which involves (a) assaying the expression of a polypeptide of the present invention in cells  
25 or body fluid of an individual; (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed

polypeptide gene expression level compared to the standard expression level is indicative of a disorder. With respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for  
5 detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

Moreover, polypeptides of the present invention can be used to treat, prevent,  
10 and/or diagnose disease. For example, patients can be administered a polypeptide of the present invention in an effort to replace absent or decreased levels of the polypeptide (e.g., insulin), to supplement absent or decreased levels of a different polypeptide (e.g., hemoglobin S for hemoglobin B, SOD, catalase, DNA repair proteins), to inhibit the activity of a polypeptide (e.g., an oncogene or tumor  
15 suppressor), to activate the activity of a polypeptide (e.g., by binding to a receptor), to reduce the activity of a membrane bound receptor by competing with it for free ligand (e.g., soluble TNF receptors used in reducing inflammation), or to bring about a desired response (e.g., blood vessel growth inhibition, enhancement of the immune response to proliferative cells or tissues).

20 Similarly, antibodies directed to a polypeptide of the present invention can also be used to treat, prevent, and/or diagnose disease. For example, administration of an antibody directed to a polypeptide of the present invention can bind and reduce overproduction of the polypeptide. Similarly, administration of an antibody can activate the polypeptide, such as by binding to a polypeptide bound to a membrane  
25 (receptor).

At the very least, the polypeptides of the present invention can be used as molecular weight markers on SDS-PAGE gels or on molecular sieve gel filtration columns using methods well known to those of skill in the art. Polypeptides can also be used to raise antibodies, which in turn are used to measure protein expression from a recombinant cell, as a way of assessing transformation of the host cell. Moreover, the polypeptides of the present invention can be used to test the following biological activities.

#### Gene Therapy Methods

Another aspect of the present invention is to gene therapy methods for treating or preventing disorders, diseases and conditions. The gene therapy methods relate to the introduction of nucleic acid (DNA, RNA and antisense DNA or RNA) sequences into an animal to achieve expression of a polypeptide of the present invention. This method requires a polynucleotide which codes for a polypeptide of the invention that operatively linked to a promoter and any other genetic elements necessary for the expression of the polypeptide by the target tissue. Such gene therapy and delivery techniques are known in the art, see, for example, WO90/11092, which is herein incorporated by reference.

Thus, for example, cells from a patient may be engineered with a polynucleotide (DNA or RNA) comprising a promoter operably linked to a polynucleotide of the invention *ex vivo*, with the engineered cells then being provided to a patient to be treated with the polypeptide. Such methods are well-known in the art. For example, see Belldegrun et al., J. Natl. Cancer Inst., 85:207-216 (1993); Ferrantini et al., Cancer Research, 53:107-1112 (1993); Ferrantini et al., J. Immunology 153: 4604-4615 (1994); Kaido, T., et al., Int. J. Cancer 60: 221-229 (1995); Ogura et al., Cancer Research 50: 5102-5106 (1990); Santodonato, et al.,

Human Gene Therapy 7:1-10 (1996); Santodonato, et al., Gene Therapy 4:1246-1255 (1997); and Zhang, et al., Cancer Gene Therapy 3: 31-38 (1996)), which are herein incorporated by reference. In one embodiment, the cells which are engineered are arterial cells. The arterial cells may be reintroduced into the patient through direct  
5 injection to the artery, the tissues surrounding the artery, or through catheter injection.

As discussed in more detail below, the polynucleotide constructs can be delivered by any method that delivers injectable materials to the cells of an animal, such as, injection into the interstitial space of tissues (heart, muscle, skin, lung, liver, and the like). The polynucleotide constructs may be delivered in a pharmaceutically  
10 acceptable liquid or aqueous carrier.

In one embodiment, the polynucleotide of the invention is delivered as a naked polynucleotide. The term "naked" polynucleotide, DNA or RNA refers to sequences that are free from any delivery vehicle that acts to assist, promote or facilitate entry into the cell, including viral sequences, viral particles, liposome formulations,  
15 lipofectin or precipitating agents and the like. However, the polynucleotides of the invention can also be delivered in liposome formulations and lipofectin formulations and the like can be prepared by methods well known to those skilled in the art. Such methods are described, for example, in U.S. Patent Nos. 5,593,972, 5,589,466, and 5,580,859, which are herein incorporated by reference.

20 The polynucleotide vector constructs of the invention used in the gene therapy method are preferably constructs that will not integrate into the host genome nor will they contain sequences that allow for replication. Appropriate vectors include pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; pSVK3, pBPV, pMSG and pSVL available from Pharmacia; and pEF1/V5,  
25 pcDNA3.1, and pRc/CMV2 available from Invitrogen. Other suitable vectors will be readily apparent to the skilled artisan.



Any strong promoter known to those skilled in the art can be used for driving the expression of polynucleotide sequence of the invention. Suitable promoters include adenoviral promoters, such as the adenoviral major late promoter; or heterologous promoters, such as the cytomegalovirus (CMV) promoter; the respiratory syncytial virus (RSV) promoter; inducible promoters, such as the MMT promoter, the metallothionein promoter; heat shock promoters; the albumin promoter; the ApoA1 promoter; human globin promoters; viral thymidine kinase promoters, such as the Herpes Simplex thymidine kinase promoter; retroviral LTRs; the  $\beta$ -actin promoter; and human growth hormone promoters. The promoter also may be the native promoter for the polynucleotides of the invention.

Unlike other gene therapy techniques, one major advantage of introducing naked nucleic acid sequences into target cells is the transitory nature of the polynucleotide synthesis in the cells. Studies have shown that non-replicating DNA sequences can be introduced into cells to provide production of the desired polypeptide for periods of up to six months.

The polynucleotide construct of the invention can be delivered to the interstitial space of tissues within the an animal, including of muscle, skin, brain, lung, liver, spleen, bone marrow, thymus, heart, lymph, blood, bone, cartilage, pancreas, kidney, gall bladder, stomach, intestine, testis, ovary, uterus, rectum, nervous system, eye, gland, and connective tissue. Interstitial space of the tissues comprises the intercellular, fluid, mucopolysaccharide matrix among the reticular fibers of organ tissues, elastic fibers in the walls of vessels or chambers, collagen fibers of fibrous tissues, or that same matrix within connective tissue ensheathing muscle cells or in the lacunae of bone. It is similarly the space occupied by the plasma of the circulation and the lymph fluid of the lymphatic channels. Delivery to the interstitial space of muscle tissue is preferred for the reasons discussed below. They may be conveniently delivered by

injection into the tissues comprising these cells. They are preferably delivered to and expressed in persistent, non-dividing cells which are differentiated, although delivery and expression may be achieved in non-differentiated or less completely differentiated cells, such as, for example, stem cells of blood or skin fibroblasts. *In vivo* muscle cells  
5 are particularly competent in their ability to take up and express polynucleotides.

For the naked *nucleic acid* sequence injection, an effective dosage amount of DNA or RNA will be in the range of from about 0.05 mg/kg body weight to about 50 mg/kg body weight. Preferably the dosage will be from about 0.005 mg/kg to about 20 mg/kg and more preferably from about 0.05 mg/kg to about 5 mg/kg. Of course, as  
10 the artisan of ordinary skill will appreciate, this dosage will vary according to the tissue site of injection. The appropriate and effective dosage of nucleic acid sequence can readily be determined by those of ordinary skill in the art and may depend on the condition being treated and the route of administration.

The preferred route of administration is by the parenteral route of injection  
15 into the interstitial space of tissues. However, other parenteral routes may also be used, such as, inhalation of an aerosol formulation particularly for delivery to lungs or bronchial tissues, throat or mucous membranes of the nose. In addition, naked DNA constructs can be delivered to arteries during angioplasty by the catheter used in the procedure.

20 The naked polynucleotides are delivered by any method known in the art, including, but not limited to, direct needle injection at the delivery site, intravenous injection, topical administration, catheter infusion, and so-called "gene guns". These delivery methods are known in the art.

The constructs may also be delivered with delivery vehicles such as viral  
25 sequences, viral particles, liposome formulations, lipofectin, precipitating agents, etc. Such methods of delivery are known in the art.

In certain embodiments, the polynucleotide constructs of the invention are complexed in a liposome preparation. Liposomal preparations for use in the instant invention include cationic (positively charged), anionic (negatively charged) and neutral preparations. However, cationic liposomes are particularly preferred because a  
5 tight charge complex can be formed between the cationic liposome and the polyanionic nucleic acid. Cationic liposomes have been shown to mediate intracellular delivery of plasmid DNA (Felgner et al., Proc. Natl. Acad. Sci. USA , 84:7413-7416 (1987), which is herein incorporated by reference); mRNA (Malone et al., Proc. Natl. Acad. Sci. USA , 86:6077-6081 (1989), which is herein incorporated  
10 by reference); and purified transcription factors (Debs et al., J. Biol. Chem., 265:10189-10192 (1990), which is herein incorporated by reference), in functional form.

Cationic liposomes are readily available. For example, N[1-2,3-dioleoyloxy)propyl]-N,N,N-triethylammonium (DOTMA) liposomes are  
15 particularly useful and are available under the trademark Lipofectin, from GIBCO BRL, Grand Island, N.Y. (See, also, Felgner et al., Proc. Natl Acad. Sci. USA , 84:7413-7416 (1987), which is herein incorporated by reference). Other commercially available liposomes include transfectace (DDAB/DOPE) and DOTAP/DOPE (Boehringer).

20 Other cationic liposomes can be prepared from readily available materials using techniques well known in the art. See, e.g. PCT Publication NO: WO 90/11092 (which is herein incorporated by reference) for a description of the synthesis of DOTAP (1,2-bis(oleoyloxy)-3-(trimethylammonio)propane) liposomes. Preparation of DOTMA liposomes is explained in the literature, see, e.g., Felgner et al., Proc.  
25 Natl. Acad. Sci. USA, 84:7413-7417, which is herein incorporated by reference. Similar methods can be used to prepare liposomes from other cationic lipid materials.

Similarly, anionic and neutral liposomes are readily available, such as from Avanti Polar Lipids (Birmingham, Ala.), or can be easily prepared using readily available materials. Such materials include phosphatidyl, choline, cholesterol, phosphatidyl ethanolamine, dioleoylphosphatidyl choline (DOPC),  
5 dioleoylphosphatidyl glycerol (DOPG), dioleoylphosphatidyl ethanolamine (DOPE), among others. These materials can also be mixed with the DOTMA and DOTAP starting materials in appropriate ratios. Methods for making liposomes using these materials are well known in the art.

For example, commercially dioleoylphosphatidyl choline (DOPC),  
10 dioleoylphosphatidyl glycerol (DOPG), and dioleoylphosphatidyl ethanolamine (DOPE) can be used in various combinations to make conventional liposomes, with or without the addition of cholesterol. Thus, for example, DOPG/DOPC vesicles can be prepared by drying 50 mg each of DOPG and DOPC under a stream of nitrogen gas into a sonication vial. The sample is placed under a vacuum pump overnight and is  
15 hydrated the following day with deionized water. The sample is then sonicated for 2 hours in a capped vial, using a Heat Systems model 350 sonicator equipped with an inverted cup (bath type) probe at the maximum setting while the bath is circulated at 15EC. Alternatively, negatively charged vesicles can be prepared without sonication to produce multilamellar vesicles or by extrusion through nucleopore membranes to  
20 produce unilamellar vesicles of discrete size. Other methods are known and available to those of skill in the art.

The liposomes can comprise multilamellar vesicles (MLVs), small unilamellar vesicles (SUVs), or large unilamellar vesicles (LUVs), with SUVs being preferred. The various liposome-nucleic acid complexes are prepared using methods well known  
25 in the art. See, e.g., Straubinger et al., *Methods of Immunology*, 101:512-527 (1983), which is herein incorporated by reference. For example, MLVs containing nucleic

acid can be prepared by depositing a thin film of phospholipid on the walls of a glass tube and subsequently hydrating with a solution of the material to be encapsulated.

SUVs are prepared by extended sonication of MLVs to produce a homogeneous population of unilamellar liposomes. The material to be entrapped is added to a

5 suspension of preformed MLVs and then sonicated. When using liposomes containing cationic lipids, the dried lipid film is resuspended in an appropriate solution such as sterile water or an isotonic buffer solution such as 10 mM Tris/NaCl, sonicated, and then the preformed liposomes are mixed directly with the DNA. The liposome and DNA form a very stable complex due to binding of the positively charged liposomes  
10 to the cationic DNA. SUVs find use with small nucleic acid fragments. LUVs are prepared by a number of methods, well known in the art. Commonly used methods include  $\text{Ca}^{2+}$ -EDTA chelation (Papahadjopoulos et al., *Biochim. Biophys. Acta*, 394:483 (1975); Wilson et al., *Cell*, 17:77 (1979)); ether injection (Deamer et al., *Biochim. Biophys. Acta*, 443:629 (1976); Ostro et al., *Biochem. Biophys. Res.*  
15 *Commun.*, 76:836 (1977); Fraley et al., *Proc. Natl. Acad. Sci. USA*, 76:3348 (1979)); detergent dialysis (Enoch et al., *Proc. Natl. Acad. Sci. USA*, 76:145 (1979)); and reverse-phase evaporation (REV) (Fraley et al., *J. Biol. Chem.*, 255:10431 (1980); Szoka et al., *Proc. Natl. Acad. Sci. USA*, 75:145 (1978); Schaefer-Ridder et al., *Science*, 215:166 (1982)), which are herein incorporated by reference.

20 Generally, the ratio of DNA to liposomes will be from about 10:1 to about 1:10. Preferably, the ration will be from about 5:1 to about 1:5. More preferably, the ration will be about 3:1 to about 1:3. Still more preferably, the ratio will be about 1:1.

U.S. Patent NO: 5,676,954 (which is herein incorporated by reference) reports on the injection of genetic material, complexed with cationic liposomes carriers, into  
25 mice. U.S. Patent Nos. 4,897,355, 4,946,787, 5,049,386, 5,459,127, 5,589,466, 5,693,622, 5,580,859, 5,703,055, and international publication NO: WO 94/9469

(which are herein incorporated by reference) provide cationic lipids for use in transfecting DNA into cells and mammals. U.S. Patent Nos. 5,589,466, 5,693,622, 5,580,859, 5,703,055, and international publication NO: WO 94/9469 (which are herein incorporated by reference) provide methods for delivering DNA-cationic lipid  
5 complexes to mammals.

In certain embodiments, cells are engineered, *ex vivo* or *in vivo*, using a retroviral particle containing RNA which comprises a sequence encoding polypeptides of the invention. Retroviruses from which the retroviral plasmid vectors may be derived include, but are not limited to, Moloney Murine Leukemia Virus,  
10 spleen necrosis virus, Rous sarcoma Virus, Harvey Sarcoma Virus, avian leukosis virus, gibbon ape leukemia virus, human immunodeficiency virus, Myeloproliferative Sarcoma Virus, and mammary tumor virus.

The retroviral plasmid vector is employed to transduce packaging cell lines to form producer cell lines. Examples of packaging cells which may be transfected  
15 include, but are not limited to, the PE501, PA317, R-2, R-AM, PA12, T19-14X, VT-19-17-H2, RCRE, RCRIP, GP+E-86, GP+envAm12, and DAN cell lines as described in Miller, Human Gene Therapy , 1:5-14 (1990), which is incorporated herein by reference in its entirety. The vector may transduce the packaging cells through any means known in the art. Such means include, but are not limited to, electroporation,  
20 the use of liposomes, and CaPO<sub>4</sub> precipitation. In one alternative, the retroviral plasmid vector may be encapsulated into a liposome, or coupled to a lipid, and then administered to a host.

The producer cell line generates infectious retroviral vector particles which include polynucleotide encoding polypeptides of the invention. Such retroviral vector  
25 particles then may be employed, to transduce eukaryotic cells, either *in vitro* or *in vivo*. The transduced eukaryotic cells will express polypeptides of the invention.

In certain other embodiments, cells are engineered, *ex vivo* or *in vivo*, with polynucleotides of the invention contained in an adenovirus vector. Adenovirus can be manipulated such that it encodes and expresses polypeptides of the invention, and at the same time is inactivated in terms of its ability to replicate in a normal lytic viral life cycle. Adenovirus expression is achieved without integration of the viral DNA into the host cell chromosome, thereby alleviating concerns about insertional mutagenesis. Furthermore, adenoviruses have been used as live enteric vaccines for many years with an excellent safety profile (Schwartz et al., Am. Rev. Respir. Dis., 109:233-238 (1974)). Finally, adenovirus mediated gene transfer has been demonstrated in a number of instances including transfer of alpha-1-antitrypsin and CFTR to the lungs of cotton rats (Rosenfeld et al., Science, 252:431-434 (1991); Rosenfeld et al., Cell, 68:143-155 (1992)). Furthermore, extensive studies to attempt to establish adenovirus as a causative agent in human cancer were uniformly negative (Green et al. Proc. Natl. Acad. Sci. USA, 76:6606 (1979)).

Suitable adenoviral vectors useful in the present invention are described, for example, in Kozarsky and Wilson, Curr. Opin. Genet. Devel., 3:499-503 (1993); Rosenfeld et al., Cell, 68:143-155 (1992); Engelhardt et al., Human Genet. Ther., 4:759-769 (1993); Yang et al., Nature Genet., 7:362-369 (1994); Wilson et al., Nature, 365:691-692 (1993); and U.S. Patent NO: 5,652,224, which are herein incorporated by reference. For example, the adenovirus vector Ad2 is useful and can be grown in human 293 cells. These cells contain the E1 region of adenovirus and constitutively express E1a and E1b, which complement the defective adenoviruses by providing the products of the genes deleted from the vector. In addition to Ad2, other varieties of adenovirus (e.g., Ad3, Ad5, and Ad7) are also useful in the present invention.

Preferably, the adenoviruses used in the present invention are replication deficient. Replication deficient adenoviruses require the aid of a helper virus and/or packaging cell line to form infectious particles. The resulting virus is capable of infecting cells and can express a polynucleotide of interest which is operably linked to a promoter, but cannot replicate in most cells. Replication deficient adenoviruses may be deleted in one or more of all or a portion of the following genes: E1a, E1b, E3, E4, E2a, or L1 through L5.

In certain other embodiments, the cells are engineered, *ex vivo* or *in vivo*, using an adeno-associated virus (AAV). AAVs are naturally occurring defective viruses that require helper viruses to produce infectious particles (Muzyczka, Curr. Topics in Microbiol. Immunol., 158:97 (1992)). It is also one of the few viruses that may integrate its DNA into non-dividing cells. Vectors containing as little as 300 base pairs of AAV can be packaged and can integrate, but space for exogenous DNA is limited to about 4.5 kb. Methods for producing and using such AAVs are known in the art. See, for example, U.S. Patent Nos. 5,139,941, 5,173,414, 5,354,678, 5,436,146, 5,474,935, 5,478,745, and 5,589,377.

For example, an appropriate AAV vector for use in the present invention will include all the sequences necessary for DNA replication, encapsidation, and host-cell integration. The polynucleotide construct containing polynucleotides of the invention is inserted into the AAV vector using standard cloning methods, such as those found in Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press (1989). The recombinant AAV vector is then transfected into packaging cells which are infected with a helper virus, using any standard technique, including lipofection, electroporation, calcium phosphate precipitation, etc. Appropriate helper viruses include adenoviruses, cytomegaloviruses, vaccinia viruses, or herpes viruses. Once the packaging cells are transfected and infected, they will produce infectious



AAV viral particles which contain the polynucleotide construct of the invention.

These viral particles are then used to transduce eukaryotic cells, either *ex vivo* or *in vivo*. The transduced cells will contain the polynucleotide construct integrated into its genome, and will express the desired gene product.

- 5           Another method of gene therapy involves operably associating heterologous control regions and endogenous polynucleotide sequences (e.g. encoding the polypeptide sequence of interest) via homologous recombination (see, e.g., U.S. Patent NO: 5,641,670, issued June 24, 1997; International Publication NO: WO 96/29411, published September 26, 1996; International Publication NO: WO 10 94/12650, published August 4, 1994; Koller et al., Proc. Natl. Acad. Sci. USA, 86:8932-8935 (1989); and Zijlstra et al., Nature, 342:435-438 (1989). This method involves the activation of a gene which is present in the target cells, but which is not normally expressed in the cells, or is expressed at a lower level than desired.

- Polynucleotide constructs are made, using standard techniques known in the art, which contain the promoter with targeting sequences flanking the promoter. 15           Suitable promoters are described herein. The targeting sequence is sufficiently complementary to an endogenous sequence to permit homologous recombination of the promoter-targeting sequence with the endogenous sequence. The targeting sequence will be sufficiently near the 5' end of the desired endogenous 20 polynucleotide sequence so the promoter will be operably linked to the endogenous sequence upon homologous recombination.

- The promoter and the targeting sequences can be amplified using PCR. Preferably, the amplified promoter contains distinct restriction enzyme sites on the 5' and 3' ends. Preferably, the 3' end of the first targeting sequence contains the same 25 restriction enzyme site as the 5' end of the amplified promoter and the 5' end of the second targeting sequence contains the same restriction site as the 3' end of the

amplified promoter. The amplified promoter and targeting sequences are digested and ligated together.

The promoter-targeting sequence construct is delivered to the cells, either as naked polynucleotide, or in conjunction with transfection-facilitating agents, such as liposomes, viral sequences, viral particles, whole viruses, lipofection, precipitating agents, etc., described in more detail above. The P promoter-targeting sequence can be delivered by any method, included direct needle injection, intravenous injection, topical administration, catheter infusion, particle accelerators, etc. The methods are described in more detail below.

10 The promoter-targeting sequence construct is taken up by cells. Homologous recombination between the construct and the endogenous sequence takes place, such that an endogenous sequence is placed under the control of the promoter. The promoter then drives the expression of the endogenous sequence.

The polynucleotides encoding polypeptides of the present invention may be administered along with other polynucleotides encoding other angiogenic proteins. Angiogenic proteins include, but are not limited to, acidic and basic fibroblast growth factors, VEGF-1, VEGF-2 (VEGF-C), VEGF-3 (VEGF-B), epidermal growth factor alpha and beta, platelet-derived endothelial cell growth factor, platelet-derived growth factor, tumor necrosis factor alpha, hepatocyte growth factor, insulin like growth factor, colony stimulating factor, macrophage colony stimulating factor, granulocyte/macrophage colony stimulating factor, and nitric oxide synthase.

20 Preferably, the polynucleotide encoding a polypeptide of the invention contains a secretory signal sequence that facilitates secretion of the protein. Typically, the signal sequence is positioned in the coding region of the polynucleotide to be expressed towards or at the 5' end of the coding region. The signal sequence may be homologous or heterologous to the polynucleotide of interest and may be

homologous or heterologous to the cells to be transfected. Additionally, the signal sequence may be chemically synthesized using methods known in the art.

Any mode of administration of any of the above-described polynucleotides constructs can be used so long as the mode results in the expression of one or more molecules in an amount sufficient to provide a therapeutic effect. This includes direct  
5 needle injection, systemic injection, catheter infusion, biolistic injectors, particle accelerators (i.e., "gene guns"), gelfoam sponge depots, other commercially available depot materials, osmotic pumps (e.g., Alza minipumps), oral or suppositorial solid (tablet or pill) pharmaceutical formulations, and decanting or topical applications  
10 during surgery. For example, direct injection of naked calcium phosphate-precipitated plasmid into rat liver and rat spleen or a protein-coated plasmid into the portal vein has resulted in gene expression of the foreign gene in the rat livers. (Kaneda et al., Science, 243:375 (1989)).

A preferred method of local administration is by direct injection. Preferably, a  
15 recombinant molecule of the present invention complexed with a delivery vehicle is administered by direct injection into or locally within the area of arteries. Administration of a composition locally within the area of arteries refers to injecting the composition centimeters and preferably, millimeters within arteries.

Another method of local administration is to contact a polynucleotide  
20 construct of the present invention in or around a surgical wound. For example, a patient can undergo surgery and the polynucleotide construct can be coated on the surface of tissue inside the wound or the construct can be injected into areas of tissue inside the wound.

Therapeutic compositions useful in systemic administration, include  
25 recombinant molecules of the present invention complexed to a targeted delivery vehicle of the present invention. Suitable delivery vehicles for use with systemic

administration comprise liposomes comprising ligands for targeting the vehicle to a particular site.

Preferred methods of systemic administration, include intravenous injection, aerosol, oral and percutaneous (topical) delivery. Intravenous injections can be performed using methods standard in the art. Aerosol delivery can also be performed using methods standard in the art (see, for example, Stribling et al., Proc. Natl. Acad. Sci. USA , 189:11277-11281 (1992), which is incorporated herein by reference). Oral delivery can be performed by complexing a polynucleotide construct of the present invention to a carrier capable of withstanding degradation by digestive enzymes in the gut of an animal. Examples of such carriers, include plastic capsules or tablets, such as those known in the art. Topical delivery can be performed by mixing a polynucleotide construct of the present invention with a lipophilic reagent (e.g., DMSO) that is capable of passing into the skin.

Determining an effective amount of substance to be delivered can depend upon a number of factors including, for example, the chemical structure and biological activity of the substance, the age and weight of the animal, the precise condition requiring treatment and its severity, and the route of administration. The frequency of treatments depends upon a number of factors, such as the amount of polynucleotide constructs administered per dose, as well as the health and history of the subject. The precise amount, number of doses, and timing of doses will be determined by the attending physician or veterinarian. Therapeutic compositions of the present invention can be administered to any animal, preferably to mammals and birds. Preferred mammals include humans, dogs, cats, mice, rats, rabbits sheep, cattle, horses and pigs, with humans being particularly

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### **Biological Activities**

The polynucleotides or polypeptides, or agonists or antagonists of the present invention can be used in assays to test for one or more biological activities. If these polynucleotides and polypeptides do exhibit activity in a particular assay, it is likely that these molecules may be involved in the diseases associated with the biological activity. Thus, the polynucleotides or polypeptides, or agonists or antagonists could be used to treat the associated disease.

### **Immune Activity**

The polynucleotides or polypeptides, or agonists or antagonists of the present invention may be useful in treating, preventing, and/or diagnosing diseases, disorders, and/or conditions of the immune system, by activating or inhibiting the proliferation, differentiation, or mobilization (chemotaxis) of immune cells. Immune cells develop through a process called hematopoiesis, producing myeloid (platelets, red blood cells, neutrophils, and macrophages) and lymphoid (B and T lymphocytes) cells from pluripotent stem cells. The etiology of these immune diseases, disorders, and/or conditions may be genetic, somatic, such as cancer or some autoimmune diseases, disorders, and/or conditions, acquired (e.g., by chemotherapy or toxins), or infectious. Moreover, a polynucleotides or polypeptides, or agonists or antagonists of the present invention can be used as a marker or detector of a particular immune system disease or disorder.

A polynucleotides or polypeptides, or agonists or antagonists of the present invention may be useful in treating, preventing, and/or diagnosing diseases, disorders, and/or conditions of hematopoietic cells. A polynucleotides or polypeptides, or agonists or antagonists of the present invention could be used to increase differentiation and proliferation of hematopoietic cells, including the pluripotent stem cells, in an effort to treat or prevent those diseases, disorders, and/or conditions

associated with a decrease in certain (or many) types hematopoietic cells. Examples of immunologic deficiency syndromes include, but are not limited to: blood protein diseases, disorders, and/or conditions (e.g. agammaglobulinemia, dysgammaglobulinemia), ataxia telangiectasia, common variable immunodeficiency,

- 5    Digeorge Syndrome, HIV infection, HTLV-BLV infection, leukocyte adhesion deficiency syndrome, lymphopenia, phagocyte bactericidal dysfunction, severe combined immunodeficiency (SCIDs), Wiskott-Aldrich Disorder, anemia, thrombocytopenia, or hemoglobinuria.

- 10    Moreover, a polynucleotides or polypeptides, or agonists or antagonists of the present invention could also be used to modulate hemostatic (the stopping of bleeding) or thrombolytic activity (clot formation). For example, by increasing hemostatic or thrombolytic activity, a polynucleotides or polypeptides, or agonists or antagonists of the present invention could be used to treat or prevent blood coagulation diseases, disorders, and/or conditions (e.g., afibrinogenemia, factor
- 15    deficiencies), blood platelet diseases, disorders, and/or conditions (e.g. thrombocytopenia), or wounds resulting from trauma, surgery, or other causes.

- Alternatively, a polynucleotides or polypeptides, or agonists or antagonists of the present invention that can decrease hemostatic or thrombolytic activity could be used to inhibit or dissolve clotting. These molecules could be important in the treatment or
- 20    prevention of heart attacks (infarction), strokes, or scarring.

- A polynucleotides or polypeptides, or agonists or antagonists of the present invention may also be useful in treating, preventing, and/or diagnosing autoimmune diseases, disorders, and/or conditions. Many autoimmune diseases, disorders, and/or conditions result from inappropriate recognition of self as foreign material by immune
- 25    cells. This inappropriate recognition results in an immune response leading to the destruction of the host tissue. Therefore, the administration of a polynucleotides or

polypeptides, or agonists or antagonists of the present invention that inhibits an immune response, particularly the proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing autoimmune diseases, disorders, and/or conditions.

5           Examples of autoimmune diseases, disorders, and/or conditions that can be treated, prevented, and/or diagnosed or detected by the present invention include, but are not limited to: Addison's Disease, hemolytic anemia, antiphospholipid syndrome, rheumatoid arthritis, dermatitis, allergic encephalomyelitis, glomerulonephritis, Goodpasture's Syndrome, Graves' Disease, Multiple Sclerosis, Myasthenia Gravis,  
10   Neuritis, Ophthalmia, Bullous Pemphigoid, Pemphigus, Polyendocrinopathies, Purpura, Reiter's Disease, Stiff-Man Syndrome, Autoimmune Thyroiditis, Systemic Lupus Erythematosus, Autoimmune Pulmonary Inflammation, Guillain-Barre Syndrome, insulin dependent diabetes mellitus, and autoimmune inflammatory eye disease.

15           Similarly, allergic reactions and conditions, such as asthma (particularly allergic asthma) or other respiratory problems, may also be treated, prevented, and/or diagnosed by polynucleotides or polypeptides, or agonists or antagonists of the present invention. Moreover, these molecules can be used to treat anaphylaxis, hypersensitivity to an antigenic molecule, or blood group incompatibility.

20           A polynucleotides or polypeptides, or agonists or antagonists of the present invention may also be used to treat, prevent, and/or diagnose organ rejection or graft-versus-host disease (GVHD). Organ rejection occurs by host immune cell destruction of the transplanted tissue through an immune response. Similarly, an immune response is also involved in GVHD, but, in this case, the foreign transplanted immune  
25   cells destroy the host tissues. The administration of a polynucleotides or polypeptides, or agonists or antagonists of the present invention that inhibits an

immune response, particularly the proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing organ rejection or GVHD.

Similarly, a polynucleotides or polypeptides, or agonists or antagonists of the present invention may also be used to modulate inflammation. For example, the polypeptide or polynucleotide or agonists or antagonist may inhibit the proliferation and differentiation of cells involved in an inflammatory response. These molecules can be used to treat, prevent, and/or diagnose inflammatory conditions, both chronic and acute conditions, including chronic prostatitis, granulomatous prostatitis and malacoplakia, inflammation associated with infection (e.g., septic shock, sepsis, or systemic inflammatory response syndrome (SIRS)), ischemia-reperfusion injury, endotoxin lethality, arthritis, complement-mediated hyperacute rejection, nephritis, cytokine or chemokine induced lung injury, inflammatory bowel disease, Crohn's disease, or resulting from over production of cytokines (e.g., TNF or IL-1.)

#### 15 **Hyperproliferative Disorders**

A polynucleotides or polypeptides, or agonists or antagonists of the invention can be used to treat, prevent, and/or diagnose hyperproliferative diseases, disorders, and/or conditions, including neoplasms. A polynucleotides or polypeptides, or agonists or antagonists of the present invention may inhibit the proliferation of the disorder through direct or indirect interactions. Alternatively, a polynucleotides or polypeptides, or agonists or antagonists of the present invention may proliferate other cells which can inhibit the hyperproliferative disorder.

For example, by increasing an immune response, particularly increasing antigenic qualities of the hyperproliferative disorder or by proliferating, differentiating, or mobilizing T-cells, hyperproliferative diseases, disorders, and/or conditions can be treated, prevented, and/or diagnosed. This immune response may



be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively, decreasing an immune response may also be a method of treating, preventing, and/or diagnosing hyperproliferative diseases, disorders, and/or conditions, such as a chemotherapeutic agent.

- 5           Examples of hyperproliferative diseases, disorders, and/or conditions that can be treated, prevented, and/or diagnosed by polynucleotides or polypeptides, or agonists or antagonists of the present invention include, but are not limited to neoplasms located in the: colon, abdomen, bone, breast, digestive system, liver, pancreas, peritoneum, endocrine glands (adrenal, parathyroid, pituitary, testicles, 10   ovary, thymus, thyroid), eye, head and neck, nervous (central and peripheral), lymphatic system, pelvic, skin, soft tissue, spleen, thoracic, and urogenital.

- Similarly, other hyperproliferative diseases, disorders, and/or conditions can also be treated, prevented, and/or diagnosed by a polynucleotides or polypeptides, or agonists or antagonists of the present invention. Examples of such hyperproliferative 15   diseases, disorders, and/or conditions include, but are not limited to: hypergammaglobulinemia, lymphoproliferative diseases, disorders, and/or conditions, paraproteinemias, purpura, sarcoidosis, Sezary Syndrome, Waldenström's Macroglobulinemia, Gaucher's Disease, histiocytosis, and any other hyperproliferative disease, besides neoplasia, located in an organ system listed above.

- 20           One preferred embodiment utilizes polynucleotides of the present invention to inhibit aberrant cellular division, by gene therapy using the present invention, and/or protein fusions or fragments thereof.

- Thus, the present invention provides a method for treating or preventing cell proliferative diseases, disorders, and/or conditions by inserting into an abnormally 25   proliferating cell a polynucleotide of the present invention, wherein said polynucleotide represses said expression.

Another embodiment of the present invention provides a method of treating or preventing cell-proliferative diseases, disorders, and/or conditions in individuals comprising administration of one or more active gene copies of the present invention to an abnormally proliferating cell or cells. In a preferred embodiment, polynucleotides of the present invention is a DNA construct comprising a recombinant expression vector effective in expressing a DNA sequence encoding said polynucleotides. In another preferred embodiment of the present invention, the DNA construct encoding the polynucleotides of the present invention is inserted into cells to be treated utilizing a retrovirus, or more preferably an adenoviral vector (See G J. Nabel, et. al., PNAS 1999 96: 324-326, which is hereby incorporated by reference). In a most preferred embodiment, the viral vector is defective and will not transform non-proliferating cells, only proliferating cells. Moreover, in a preferred embodiment, the polynucleotides of the present invention inserted into proliferating cells either alone, or in combination with or fused to other polynucleotides, can then be modulated via an external stimulus (i.e. magnetic, specific small molecule, chemical, or drug administration, etc.), which acts upon the promoter upstream of said polynucleotides to induce expression of the encoded protein product. As such the beneficial therapeutic affect of the present invention may be expressly modulated (i.e. to increase, decrease, or inhibit expression of the present invention) based upon said external stimulus.

Polynucleotides of the present invention may be useful in repressing expression of oncogenic genes or antigens. By "repressing expression of the oncogenic genes " is intended the suppression of the transcription of the gene, the degradation of the gene transcript (pre-message RNA), the inhibition of splicing, the destruction of the messenger RNA, the prevention of the post-translational

modifications of the protein, the destruction of the protein, or the inhibition of the normal function of the protein.

For local administration to abnormally proliferating cells, polynucleotides of the present invention may be administered by any method known to those of skill in the art including, but not limited to transfection, electroporation, microinjection of  
5 cells, or in vehicles such as liposomes, lipofectin, or as naked polynucleotides, or any other method described throughout the specification. The polynucleotide of the present invention may be delivered by known gene delivery systems such as, but not limited to, retroviral vectors (Gilboa, J. Virology 44:845 (1982); Hocke, Nature  
10 320:275 (1986); Wilson, et al., Proc. Natl. Acad. Sci. U.S.A. 85:3014), vaccinia virus system (Chakrabarty et al., Mol. Cell Biol. 5:3403 (1985) or other efficient DNA delivery systems (Yates et al., Nature 313:812 (1985)) known to those skilled in the art. These references are exemplary only and are hereby incorporated by reference. In order to specifically deliver or transfect cells which are abnormally proliferating  
15 and spare non-dividing cells, it is preferable to utilize a retrovirus, or adenoviral (as described in the art and elsewhere herein) delivery system known to those of skill in the art. Since host DNA replication is required for retroviral DNA to integrate and the retrovirus will be unable to self replicate due to the lack of the retrovirus genes needed for its life cycle. Utilizing such a retroviral delivery system for  
20 polynucleotides of the present invention will target said gene and constructs to abnormally proliferating cells and will spare the non-dividing normal cells.

The polynucleotides of the present invention may be delivered directly to cell proliferative disorder/disease sites in internal organs, body cavities and the like by use of imaging devices used to guide an injecting needle directly to the disease site. The  
25 polynucleotides of the present invention may also be administered to disease sites at the time of surgical intervention.

By "cell proliferative disease" is meant any human or animal disease or disorder, affecting any one or any combination of organs, cavities, or body parts, which is characterized by single or multiple local abnormal proliferations of cells, groups of cells, or tissues, whether benign or malignant.

5 Any amount of the polynucleotides of the present invention may be administered as long as it has a biologically inhibiting effect on the proliferation of the treated cells. Moreover, it is possible to administer more than one of the polynucleotide of the present invention simultaneously to the same site. By "biologically inhibiting" is meant partial or total growth inhibition as well as  
10 decreases in the rate of proliferation or growth of the cells. The biologically inhibitory dose may be determined by assessing the effects of the polynucleotides of the present invention on target malignant or abnormally proliferating cell growth in tissue culture, tumor growth in animals and cell cultures, or any other method known to one of ordinary skill in the art.

15 The present invention is further directed to antibody-based therapies which involve administering of anti-polypeptides and anti-polynucleotide antibodies to a mammalian, preferably human, patient for treating, preventing, and/or diagnosing one or more of the described diseases, disorders, and/or conditions. Methods for producing anti-polypeptides and anti-polynucleotide antibodies polyclonal and  
20 monoclonal antibodies are described in detail elsewhere herein. Such antibodies may be provided in pharmaceutically acceptable compositions as known in the art or as described herein.

A summary of the ways in which the antibodies of the present invention may be used therapeutically includes binding polynucleotides or polypeptides of the  
25 present invention locally or systemically in the body or by direct cytotoxicity of the antibody, e.g. as mediated by complement (CDC) or by effector cells (ADCC). Some

of these approaches are described in more detail below. Armed with the teachings provided herein, one of ordinary skill in the art will know how to use the antibodies of the present invention for diagnostic, monitoring or therapeutic purposes without undue experimentation.

5 In particular, the antibodies, fragments and derivatives of the present invention are useful for treating, preventing, and/or diagnosing a subject having or developing cell proliferative and/or differentiation diseases, disorders, and/or conditions as described herein. Such treatment comprises administering a single or multiple doses of the antibody, or a fragment, derivative, or a conjugate thereof.

10 The antibodies of this invention may be advantageously utilized in combination with other monoclonal or chimeric antibodies, or with lymphokines or hematopoietic growth factors, for example, which serve to increase the number or activity of effector cells which interact with the antibodies.

It is preferred to use high affinity and/or potent in vivo inhibiting and/or  
15 neutralizing antibodies against polypeptides or polynucleotides of the present invention, fragments or regions thereof, for both immunoassays directed to and therapy of diseases, disorders, and/or conditions related to polynucleotides or polypeptides, including fragments thereof, of the present invention. Such antibodies, fragments, or regions, will preferably have an affinity for polynucleotides or  
20 polypeptides, including fragments thereof. Preferred binding affinities include those with a dissociation constant or  $K_d$  less than  $5 \times 10^{-6}M$ ,  $10^{-6}M$ ,  $5 \times 10^{-7}M$ ,  $10^{-7}M$ ,  $5 \times 10^{-8}M$ ,  $10^{-8}M$ ,  $5 \times 10^{-9}M$ ,  $10^{-9}M$ ,  $5 \times 10^{-10}M$ ,  $10^{-10}M$ ,  $5 \times 10^{-11}M$ ,  $10^{-11}M$ ,  $5 \times 10^{-12}M$ ,  $10^{-12}M$ ,  $5 \times 10^{-13}M$ ,  $10^{-13}M$ ,  $5 \times 10^{-14}M$ ,  $10^{-14}M$ ,  $5 \times 10^{-15}M$ , and  $10^{-15}M$ .

Moreover, polypeptides of the present invention are useful in inhibiting the  
25 angiogenesis of proliferative cells or tissues, either alone, as a protein fusion, or in combination with other polypeptides directly or indirectly, as described elsewhere

herein. In a most preferred embodiment, said anti-angiogenesis effect may be achieved indirectly, for example, through the inhibition of hematopoietic, tumor-specific cells, such as tumor-associated macrophages (See Joseph IB, et al. J Natl Cancer Inst, 90(21):1648-53 (1998), which is hereby incorporated by reference).

- 5 Antibodies directed to polypeptides or polynucleotides of the present invention may also result in inhibition of angiogenesis directly, or indirectly (See Witte L, et al., Cancer Metastasis Rev. 17(2):155-61 (1998), which is hereby incorporated by reference)).

- Polypeptides, including protein fusions, of the present invention, or fragments thereof may be useful in inhibiting proliferative cells or tissues through the induction of apoptosis. Said polypeptides may act either directly, or indirectly to induce apoptosis of proliferative cells and tissues, for example in the activation of a death-domain receptor, such as tumor necrosis factor (TNF) receptor-1, CD95 (Fas/APO-1), TNF-receptor-related apoptosis-mediated protein (TRAMP) and TNF-related apoptosis-inducing ligand (TRAIL) receptor-1 and -2 (See Schulze-Osthoff K, et.al., Eur J Biochem 254(3):439-59 (1998), which is hereby incorporated by reference).
- 15 Moreover, in another preferred embodiment of the present invention, said polypeptides may induce apoptosis through other mechanisms, such as in the activation of other proteins which will activate apoptosis, or through stimulating the expression of said proteins, either alone or in combination with small molecule drugs or adjuvants, such as apoptonin, galectins, thioredoxins, antiinflammatory proteins (See for example, Mutat Res 400(1-2):447-55 (1998), Med Hypotheses.50(5):423-33 (1998), Chem Biol Interact. Apr 24;111-112:23-34 (1998), J Mol Med.76(6):402-12 (1998), Int J Tissue React;20(1):3-15 (1998), which are all hereby incorporated by
- 20 reference).
- 25

Polypeptides, including protein fusions to, or fragments thereof, of the present invention are useful in inhibiting the metastasis of proliferative cells or tissues. Inhibition may occur as a direct result of administering polypeptides, or antibodies directed to said polypeptides as described elsewhere herein, or indirectly, such as  
5 activating the expression of proteins known to inhibit metastasis, for example alpha 4 integrins, (See, e.g., Curr Top Microbiol Immunol 1998;231:125-41, which is hereby incorporated by reference). Such therapeutic affects of the present invention may be achieved either alone, or in combination with small molecule drugs or adjuvants.

In another embodiment, the invention provides a method of delivering  
10 compositions containing the polypeptides of the invention (e.g., compositions containing polypeptides or polypeptide antibodies associated with heterologous polypeptides, heterologous nucleic acids, toxins, or prodrugs) to targeted cells expressing the polypeptide of the present invention. Polypeptides or polypeptide antibodies of the invention may be associated with with heterologous polypeptides,  
15 heterologous nucleic acids, toxins, or prodrugs via hydrophobic, hydrophilic, ionic and/or covalent interactions.

Polypeptides, protein fusions to, or fragments thereof, of the present invention are useful in enhancing the immunogenicity and/or antigenicity of proliferating cells or tissues, either directly, such as would occur if the polypeptides of the present  
20 invention 'vaccinated' the immune response to respond to proliferative antigens and immunogens, or indirectly, such as in activating the expression of proteins known to enhance the immune response (e.g. chemokines), to said antigens and immunogens.

#### **Cardiovascular Disorders**

Polynucleotides or polypeptides, or agonists or antagonists of the invention may be used to treat, prevent, and/or diagnose cardiovascular diseases, disorders, and/or conditions, including peripheral artery disease, such as limb ischemia.

Cardiovascular diseases, disorders, and/or conditions include cardiovascular abnormalities, such as arterio-arterial fistula, arteriovenous fistula, cerebral arteriovenous malformations, congenital heart defects, pulmonary atresia, and Scimitar Syndrome. Congenital heart defects include aortic coarctation, cor triatriatum, coronary vessel anomalies, crisscross heart, dextrocardia, patent ductus arteriosus, Ebstein's anomaly, Eisenmenger complex, hypoplastic left heart syndrome, levocardia, tetralogy of fallot, transposition of great vessels, double outlet right ventricle, tricuspid atresia, persistent truncus arteriosus, and heart septal defects, such as aortopulmonary septal defect, endocardial cushion defects, Lutembacher's Syndrome, trilog y of Fallot, ventricular heart septal defects.

Cardiovascular diseases, disorders, and/or conditions also include heart disease, such as arrhythmias, carcinoid heart disease, high cardiac output, low cardiac output, cardiac tamponade, endocarditis (including bacterial), heart aneurysm, cardiac arrest, congestive heart failure, congestive cardiomyopathy, paroxysmal dyspnea, cardiac edema, heart hypertrophy, congestive cardiomyopathy, left ventricular hypertrophy, right ventricular hypertrophy, post-infarction heart rupture, ventricular septal rupture, heart valve diseases, myocardial diseases, myocardial ischemia, pericardial effusion, pericarditis (including constrictive and tuberculous), pneumopericardium, postpericardiotomy syndrome, pulmonary heart disease, rheumatic heart disease, ventricular dysfunction, hyperemia, cardiovascular pregnancy complications, Scimitar Syndrome, cardiovascular syphilis, and cardiovascular tuberculosis.



Arrhythmias include sinus arrhythmia, atrial fibrillation, atrial flutter, bradycardia, extrasystole, Adams-Stokes Syndrome, bundle-branch block, sinoatrial block, long QT syndrome, parasystole, Lown-Ganong-Levine Syndrome, Mahaim-type pre-excitation syndrome, Wolff-Parkinson-White syndrome, sick sinus syndrome, tachycardias, and ventricular fibrillation. Tachycardias include paroxysmal tachycardia, supraventricular tachycardia, accelerated idioventricular rhythm, atrioventricular nodal reentry tachycardia, ectopic atrial tachycardia, ectopic junctional tachycardia, sinoatrial nodal reentry tachycardia, sinus tachycardia, Torsades de Pointes, and ventricular tachycardia.

Heart valve disease include aortic valve insufficiency, aortic valve stenosis, hear murmurs, aortic valve prolapse, mitral valve prolapse, tricuspid valve prolapse, mitral valve insufficiency, mitral valve stenosis, pulmonary atresia, pulmonary valve insufficiency, pulmonary valve stenosis, tricuspid atresia, tricuspid valve insufficiency, and tricuspid valve stenosis.

Myocardial diseases include alcoholic cardiomyopathy, congestive cardiomyopathy, hypertrophic cardiomyopathy, aortic subvalvular stenosis, pulmonary subvalvular stenosis, restrictive cardiomyopathy, Chagas cardiomyopathy, endocardial fibroelastosis, endomyocardial fibrosis, Kearns Syndrome, myocardial reperfusion injury, and myocarditis.

Myocardial ischemias include coronary disease, such as angina pectoris, coronary aneurysm, coronary arteriosclerosis, coronary thrombosis, coronary vasospasm, myocardial infarction and myocardial stunning.

Cardiovascular diseases also include vascular diseases such as aneurysms, angiodysplasia, angiomas, bacillary angiomas, Hippiel-Lindau Disease, Klippel-Trenaunay-Weber Syndrome, Sturge-Weber Syndrome, angioneurotic edema, aortic diseases, Takayasu's Arteritis, aortitis, Leriche's Syndrome, arterial occlusive

diseases, arteritis, enarteritis, polyarteritis nodosa, cerebrovascular diseases, disorders, and/or conditions, diabetic angiopathies, diabetic retinopathy, embolisms, thrombosis, erythromelalgia, hemorrhoids, hepatic veno-occlusive disease, hypertension, hypotension, ischemia, peripheral vascular diseases, phlebitis, pulmonary veno-occlusive disease, Raynaud's disease, CREST syndrome, retinal vein occlusion, Scimitar syndrome, superior vena cava syndrome, telangiectasia, atacia telangiectasia, hereditary hemorrhagic telangiectasia, varicocele, varicose veins, varicose ulcer, vasculitis, and venous insufficiency.

Aneurysms include dissecting aneurysms, false aneurysms, infected aneurysms, ruptured aneurysms, aortic aneurysms, cerebral aneurysms, coronary aneurysms, heart aneurysms, and iliac aneurysms.

Arterial occlusive diseases include arteriosclerosis, intermittent claudication, carotid stenosis, fibromuscular dysplasias, mesenteric vascular occlusion, Moyamoya disease, renal artery obstruction, retinal artery occlusion, and thromboangiitis obliterans.

Cerebrovascular diseases, disorders, and/or conditions include carotid artery diseases, cerebral amyloid angiopathy, cerebral aneurysm, cerebral anoxia, cerebral arteriosclerosis, cerebral arteriovenous malformation, cerebral artery diseases, cerebral embolism and thrombosis, carotid artery thrombosis, sinus thrombosis, Wallenberg's syndrome, cerebral hemorrhage, epidural hematoma, subdural hematoma, subarachnoid hemorrhage, cerebral infarction, cerebral ischemia (including transient), subclavian steal syndrome, periventricular leukomalacia, vascular headache, cluster headache, migraine, and vertebrobasilar insufficiency.

Embolisms include air embolisms, amniotic fluid embolisms, cholesterol embolisms, blue toe syndrome, fat embolisms, pulmonary embolisms, and thromboembolisms. Thrombosis include coronary thrombosis, hepatic vein

thrombosis, retinal vein occlusion, carotid artery thrombosis, sinus thrombosis, Wallenberg's syndrome, and thrombophlebitis.

Ischemia includes cerebral ischemia, ischemic colitis, compartment syndromes, anterior compartment syndrome, myocardial ischemia, reperfusion  
5 injuries, and peripheral limb ischemia. Vasculitis includes aortitis, arteritis, Behcet's Syndrome, Churg-Strauss Syndrome, mucocutaneous lymph node syndrome, thromboangiitis obliterans, hypersensitivity vasculitis, Schoenlein-Henoch purpura, allergic cutaneous vasculitis, and Wegener's granulomatosis.

Polynucleotides or polypeptides, or agonists or antagonists of the invention,  
10 are especially effective for the treatment of critical limb ischemia and coronary disease.

Polypeptides may be administered using any method known in the art, including, but not limited to, direct needle injection at the delivery site, intravenous injection, topical administration, catheter infusion, biolistic injectors, particle  
15 accelerators, gelfoam sponge depots, other commercially available depot materials, osmotic pumps, oral or suppository solid pharmaceutical formulations, decanting or topical applications during surgery, aerosol delivery. Such methods are known in the art. Polypeptides of the invention may be administered as part of a *Therapeutic*, described in more detail below. Methods of delivering polynucleotides of the  
20 invention are described in more detail herein.

#### **Anti-Angiogenesis Activity**

The naturally occurring balance between endogenous stimulators and inhibitors of angiogenesis is one in which inhibitory influences predominate.  
25 Rastinejad *et al.*, *Cell* 56:345-355 (1989). In those rare instances in which neovascularization occurs under normal physiological conditions, such as wound

healing, organ regeneration, embryonic development, and female reproductive processes, angiogenesis is stringently regulated and spatially and temporally delimited. Under conditions of pathological angiogenesis such as that characterizing solid tumor growth, these regulatory controls fail. Unregulated angiogenesis becomes pathologic and sustains progression of many neoplastic and non-neoplastic diseases. A number of serious diseases are dominated by abnormal neovascularization including solid tumor growth and metastases, arthritis, some types of eye diseases, disorders, and/or conditions, and psoriasis. See, e.g., reviews by Moses *et al.*, *Biotech.* 9:630-634 (1991); Folkman *et al.*, *N. Engl. J. Med.*, 333:1757-1763 (1995); Auerbach *et al.*, *J. Microvasc. Res.* 29:401-411 (1985); Folkman, *Advances in Cancer Research*, eds. Klein and Weinhouse, Academic Press, New York, pp. 175-203 (1985); Patz, *Am. J. Ophthalmol.* 94:715-743 (1982); and Folkman *et al.*, *Science* 221:719-725 (1983). In a number of pathological conditions, the process of angiogenesis contributes to the disease state. For example, significant data have accumulated which suggest that the growth of solid tumors is dependent on angiogenesis. Folkman and Klagsbrun, *Science* 235:442-447 (1987).

The present invention provides for treatment of diseases, disorders, and/or conditions associated with neovascularization by administration of the polynucleotides and/or polypeptides of the invention, as well as agonists or antagonists of the present invention. Malignant and metastatic conditions which can be treated with the polynucleotides and polypeptides, or agonists or antagonists of the invention include, but are not limited to, malignancies, solid tumors, and cancers described herein and otherwise known in the art (for a review of such disorders, see Fishman *et al.*, *Medicine*, 2d Ed., J. B. Lippincott Co., Philadelphia (1985)). Thus, the present invention provides a method of treating, preventing, and/or diagnosing an angiogenesis-related disease and/or disorder, comprising administering to an

individual in need thereof a therapeutically effective amount of a polynucleotide, polypeptide, antagonist and/or agonist of the invention. For example, polynucleotides, polypeptides, antagonists and/or agonists may be utilized in a variety of additional methods in order to therapeutically treat or prevent a cancer or tumor.

- 5 Cancers which may be treated, prevented, and/or diagnosed with polynucleotides, polypeptides, antagonists and/or agonists include, but are not limited to solid tumors, including prostate, lung, breast, ovarian, stomach, pancreas, larynx, esophagus, testes, liver, parotid, biliary tract, colon, rectum, cervix, uterus, endometrium, kidney, bladder, thyroid cancer; primary tumors and metastases; melanomas; glioblastoma;
- 10 Kaposi's sarcoma; leiomyosarcoma; non-small cell lung cancer; colorectal cancer; advanced malignancies; and blood born tumors such as leukemias. For example, polynucleotides, polypeptides, antagonists and/or agonists may be delivered topically, in order to treat or prevent cancers such as skin cancer, head and neck tumors, breast tumors, and Kaposi's sarcoma.

- 15 Within yet other aspects, polynucleotides, polypeptides, antagonists and/or agonists may be utilized to treat superficial forms of bladder cancer by, for example, intravesical administration. Polynucleotides, polypeptides, antagonists and/or agonists may be delivered directly into the tumor, or near the tumor site, via injection or a catheter. Of course, as the artisan of ordinary skill will appreciate, the appropriate
- 20 mode of administration will vary according to the cancer to be treated. Other modes of delivery are discussed herein.

- Polynucleotides, polypeptides, antagonists and/or agonists may be useful in treating, preventing, and/or diagnosing other diseases, disorders, and/or conditions, besides cancers, which involve angiogenesis. These diseases, disorders, and/or
- 25 conditions include, but are not limited to: benign tumors, for example hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas;

arteriosclerotic plaques; ocular angiogenic diseases, for example, diabetic retinopathy, retinopathy of prematurity, macular degeneration, corneal graft rejection, neovascular glaucoma, retrolental fibroplasia, rubeosis, retinoblastoma, uveitis and Pterygia (abnormal blood vessel growth) of the eye; rheumatoid arthritis; psoriasis; delayed wound healing; endometriosis; vasculogenesis; granulations; hypertrophic scars (keloids); nonunion fractures; scleroderma; trachoma; vascular adhesions; myocardial angiogenesis; coronary collaterals; cerebral collaterals; arteriovenous malformations; ischemic limb angiogenesis; Osler-Webber Syndrome; plaque neovascularization; telangiectasia; hemophilic joints; angiofibroma; fibromuscular dysplasia; wound granulation; Crohn's disease; and atherosclerosis.

For example, within one aspect of the present invention methods are provided for treating, preventing, and/or diagnosing hypertrophic scars and keloids, comprising the step of administering a polynucleotide, polypeptide, antagonist and/or agonist of the invention to a hypertrophic scar or keloid.

Within one embodiment of the present invention polynucleotides, polypeptides, antagonists and/or agonists are directly injected into a hypertrophic scar or keloid, in order to prevent the progression of these lesions. This therapy is of particular value in the prophylactic treatment of conditions which are known to result in the development of hypertrophic scars and keloids (e.g., burns), and is preferably initiated after the proliferative phase has had time to progress (approximately 14 days after the initial injury), but before hypertrophic scar or keloid development. As noted above, the present invention also provides methods for treating, preventing, and/or diagnosing neovascular diseases of the eye, including for example, corneal neovascularization, neovascular glaucoma, proliferative diabetic retinopathy, retrolental fibroplasia and macular degeneration.

Moreover, Ocular diseases, disorders, and/or conditions associated with

neovascularization which can be treated, prevented, and/or diagnosed with the polynucleotides and polypeptides of the present invention (including agonists and/or antagonists) include, but are not limited to: neovascular glaucoma, diabetic retinopathy, retinoblastoma, retrolental fibroplasia, uveitis, retinopathy of prematurity  
5 macular degeneration, corneal graft neovascularization, as well as other eye inflammatory diseases, ocular tumors and diseases associated with choroidal or iris neovascularization. See, e.g., reviews by Waltman *et al.*, *Am. J. Ophthalmol.* 85:704-710 (1978) and Gartner *et al.*, *Surv. Ophthalmol.* 22:291-312 (1978).

Thus, within one aspect of the present invention methods are provided for  
10 treating or preventing neovascular diseases of the eye such as corneal neovascularization (including corneal graft neovascularization), comprising the step of administering to a patient a therapeutically effective amount of a compound (as described above) to the cornea, such that the formation of blood vessels is inhibited. Briefly, the cornea is a tissue which normally lacks blood vessels. In certain  
15 pathological conditions however, capillaries may extend into the cornea from the pericorneal vascular plexus of the limbus. When the cornea becomes vascularized, it also becomes clouded, resulting in a decline in the patient's visual acuity. Visual loss may become complete if the cornea completely opacitates. A wide variety of diseases, disorders, and/or conditions can result in corneal neovascularization,  
20 including for example, corneal infections (e.g., trachoma, herpes simplex keratitis, leishmaniasis and onchocerciasis), immunological processes (e.g., graft rejection and Stevens-Johnson's syndrome), alkali burns, trauma, inflammation (of any cause), toxic and nutritional deficiency states, and as a complication of wearing contact lenses.

25 Within particularly preferred embodiments of the invention, may be prepared for topical administration in saline (combined with any of the preservatives and

antimicrobial agents commonly used in ocular preparations), and administered in eyedrop form. The solution or suspension may be prepared in its pure form and administered several times daily. Alternatively, anti-angiogenic compositions, prepared as described above, may also be administered directly to the cornea. Within  
5 preferred embodiments, the anti-angiogenic composition is prepared with a muco-adhesive polymer which binds to cornea. Within further embodiments, the anti-angiogenic factors or anti-angiogenic compositions may be utilized as an adjunct to conventional steroid therapy. Topical therapy may also be useful prophylactically in corneal lesions which are known to have a high probability of inducing an angiogenic  
10 response (such as chemical burns). In these instances the treatment, likely in combination with steroids, may be instituted immediately to help prevent subsequent complications.

Within other embodiments, the compounds described above may be injected directly into the corneal stroma by an ophthalmologist under microscopic guidance.  
15 The preferred site of injection may vary with the morphology of the individual lesion, but the goal of the administration would be to place the composition at the advancing front of the vasculature (i.e., interspersed between the blood vessels and the normal cornea). In most cases this would involve perilimbic corneal injection to "protect" the cornea from the advancing blood vessels. This method may also be utilized shortly  
20 after a corneal insult in order to prophylactically prevent corneal neovascularization. In this situation the material could be injected in the perilimbic cornea interspersed between the corneal lesion and its undesired potential limbic blood supply. Such methods may also be utilized in a similar fashion to prevent capillary invasion of transplanted corneas. In a sustained-release form injections might only be required 2-  
25 3 times per year. A steroid could also be added to the injection solution to reduce inflammation resulting from the injection itself.



Within another aspect of the present invention, methods are provided for treating or preventing neovascular glaucoma, comprising the step of administering to a patient a therapeutically effective amount of a polynucleotide, polypeptide, antagonist and/or agonist to the eye, such that the formation of blood vessels is inhibited. In one embodiment, the compound may be administered topically to the eye in order to treat or prevent early forms of neovascular glaucoma. Within other embodiments, the compound may be implanted by injection into the region of the anterior chamber angle. Within other embodiments, the compound may also be placed in any location such that the compound is continuously released into the aqueous humor. Within another aspect of the present invention, methods are provided for treating or preventing proliferative diabetic retinopathy, comprising the step of administering to a patient a therapeutically effective amount of a polynucleotide, polypeptide, antagonist and/or agonist to the eyes, such that the formation of blood vessels is inhibited.

Within particularly preferred embodiments of the invention, proliferative diabetic retinopathy may be treated by injection into the aqueous humor or the vitreous, in order to increase the local concentration of the polynucleotide, polypeptide, antagonist and/or agonist in the retina. Preferably, this treatment should be initiated prior to the acquisition of severe disease requiring photocoagulation.

Within another aspect of the present invention, methods are provided for treating or preventing retrolental fibroplasia, comprising the step of administering to a patient a therapeutically effective amount of a polynucleotide, polypeptide, antagonist and/or agonist to the eye, such that the formation of blood vessels is inhibited. The compound may be administered topically, via intravitreal injection and/or via intraocular implants.

Additionally, diseases, disorders, and/or conditions which can be treated,

prevented, and/or diagnosed with the polynucleotides, polypeptides, agonists and/or agonists include, but are not limited to, hemangioma, arthritis, psoriasis, angiofibroma, atherosclerotic plaques, delayed wound healing, granulations, hemophilic joints, hypertrophic scars, nonunion fractures, Osler-Weber syndrome,  
5 pyogenic granuloma, scleroderma, trachoma, and vascular adhesions.

Moreover, diseases, disorders, and/or conditions and/or states, which can be treated, prevented, and/or diagnosed with the the polynucleotides, polypeptides, agonists and/or agonists include, but are not limited to, solid tumors, blood born tumors such as leukemias, tumor metastasis, Kaposi's sarcoma, benign tumors, for  
10 example hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas, rheumatoid arthritis, psoriasis, ocular angiogenic diseases, for example, diabetic retinopathy, retinopathy of prematurity, macular degeneration, corneal graft rejection, neovascular glaucoma, retrolental fibroplasia, rubeosis, retinoblastoma, and uviertis, delayed wound healing, endometriosis, vasculogenesis, granulations,  
15 hypertrophic scars (keloids), nonunion fractures, scleroderma, trachoma, vascular adhesions, myocardial angiogenesis, coronary collaterals, cerebral collaterals, arteriovenous malformations, ischemic limb angiogenesis, Osler-Webber Syndrome, plaque neovascularization, telangiectasia, hemophilic joints, angiofibroma fibromuscular dysplasia, wound granulation, Crohn's disease, atherosclerosis, birth  
20 control agent by preventing vascularization required for embryo implantation controlling menstruation, diseases that have angiogenesis as a pathologic consequence such as cat scratch disease (*Rochelia minalia quintosa*), ulcers (*Helicobacter pylori*), Bartonellosis and bacillary angiomatosis.

In one aspect of the birth control method, an amount of the compound  
25 sufficient to block embryo implantation is administered before or after intercourse and fertilization have occurred, thus providing an effective method of birth control,

possibly a "morning after" method. Polynucleotides, polypeptides, agonists and/or agonists may also be used in controlling menstruation or administered as either a peritoneal lavage fluid or for peritoneal implantation in the treatment of endometriosis.

5 Polynucleotides, polypeptides, agonists and/or agonists of the present invention may be incorporated into surgical sutures in order to prevent stitch granulomas.

Polynucleotides, polypeptides, agonists and/or agonists may be utilized in a wide variety of surgical procedures. For example, within one aspect of the present invention a compositions (in the form of, for example, a spray or film) may be utilized to coat or spray an area prior to removal of a tumor, in order to isolate normal surrounding tissues from malignant tissue, and/or to prevent the spread of disease to surrounding tissues. Within other aspects of the present invention, compositions (e.g., in the form of a spray) may be delivered via endoscopic procedures in order to coat tumors, or inhibit angiogenesis in a desired locale. Within yet other aspects of the present invention, surgical meshes which have been coated with anti- angiogenic compositions of the present invention may be utilized in any procedure wherein a surgical mesh might be utilized. For example, within one embodiment of the invention a surgical mesh laden with an anti-angiogenic composition may be utilized during abdominal cancer resection surgery (e.g., subsequent to colon resection) in order to provide support to the structure, and to release an amount of the anti-angiogenic factor.

Within further aspects of the present invention, methods are provided for treating tumor excision sites, comprising administering a polynucleotide, polypeptide, agonist and/or agonist to the resection margins of a tumor subsequent to excision, such that the local recurrence of cancer and the formation of new blood vessels at the

site is inhibited. Within one embodiment of the invention, the anti-angiogenic compound is administered directly to the tumor excision site (e.g., applied by swabbing, brushing or otherwise coating the resection margins of the tumor with the anti-angiogenic compound). Alternatively, the anti-angiogenic compounds may be incorporated into known surgical pastes prior to administration. Within particularly preferred embodiments of the invention, the anti-angiogenic compounds are applied after hepatic resections for malignancy, and after neurosurgical operations.

Within one aspect of the present invention, polynucleotides, polypeptides, agonists and/or agonists may be administered to the resection margin of a wide variety of tumors, including for example, breast, colon, brain and hepatic tumors. For example, within one embodiment of the invention, anti-angiogenic compounds may be administered to the site of a neurological tumor subsequent to excision, such that the formation of new blood vessels at the site are inhibited.

The polynucleotides, polypeptides, agonists and/or agonists of the present invention may also be administered along with other anti-angiogenic factors. Representative examples of other anti-angiogenic factors include: Anti-Invasive Factor, retinoic acid and derivatives thereof, paclitaxel, Suramin, Tissue Inhibitor of Metalloproteinase-1, Tissue Inhibitor of Metalloproteinase-2, Plasminogen Activator Inhibitor-1, Plasminogen Activator Inhibitor-2, and various forms of the lighter "d group" transition metals.

Lighter "d group" transition metals include, for example, vanadium, molybdenum, tungsten, titanium, niobium, and tantalum species. Such transition metal species may form transition metal complexes. Suitable complexes of the above-mentioned transition metal species include oxo transition metal complexes.

Representative examples of vanadium complexes include oxo vanadium complexes such as vanadate and vanadyl complexes. Suitable vanadate complexes

include metavanadate and orthovanadate complexes such as, for example, ammonium metavanadate, sodium metavanadate, and sodium orthovanadate. Suitable vanadyl complexes include, for example, vanadyl acetylacetonate and vanadyl sulfate including vanadyl sulfate hydrates such as vanadyl sulfate mono- and trihydrates.

5           Representative examples of tungsten and molybdenum complexes also include oxo complexes. Suitable oxo tungsten complexes include tungstate and tungsten oxide complexes. Suitable tungstate complexes include ammonium tungstate, calcium tungstate, sodium tungstate dihydrate, and tungstic acid. Suitable tungsten oxides include tungsten (IV) oxide and tungsten (VI) oxide. Suitable oxo  
10   molybdenum complexes include molybdate, molybdenum oxide, and molybdenyl complexes. Suitable molybdate complexes include ammonium molybdate and its hydrates, sodium molybdate and its hydrates, and potassium molybdate and its hydrates. Suitable molybdenum oxides include molybdenum (VI) oxide, molybdenum (VI) oxide, and molybdic acid. Suitable molybdenyl complexes include, for example,  
15   molybdenyl acetylacetonate. Other suitable tungsten and molybdenum complexes include hydroxo derivatives derived from, for example, glycerol, tartaric acid, and sugars.

A wide variety of other anti-angiogenic factors may also be utilized within the context of the present invention. Representative examples include platelet factor 4;  
20   protamine sulphate; sulphated chitin derivatives (prepared from queen crab shells), (Murata et al., Cancer Res. 51:22-26, 1991); Sulphated Polysaccharide Peptidoglycan Complex (SP- PG) (the function of this compound may be enhanced by the presence of steroids such as estrogen, and tamoxifen citrate); Staurosporine; modulators of matrix metabolism, including for example, proline analogs, cishydroxyproline, d,L-  
25   3,4-dehydroproline, Thiaproline, alpha,alpha-dipyridyl, aminopropionitrile fumarate; 4-propyl-5-(4-pyridinyl)-2(3H)-oxazolone; Methotrexate; Mitoxantrone; Heparin;

Interferons; 2 Macroglobulin-serum; ChIMP-3 (Pavloff et al., J. Bio. Chem. 267:17321-17326, 1992); Chymostatin (Tomkinson et al., Biochem J. 286:475-480, 1992); Cyclodextrin Tetradecasulfate; Eponemycin; Camptothecin; Fumagillin (Ingber et al., Nature 348:555-557, 1990); Gold Sodium Thiomalate ("GST";  
5 Matsubara and Ziff, J. Clin. Invest. 79:1440-1446, 1987); anticollagenase-serum; alpha2-antiplasmin (Holmes et al., J. Biol. Chem. 262(4):1659-1664, 1987); Bisantrene (National Cancer Institute); Lobenzarit disodium (N-(2)-carboxyphenyl-4-chloroanthronilic acid disodium or "CCA"; Takeuchi et al., Agents Actions 36:312-316, 1992); Thalidomide; Angostatic steroid; AGM-1470; carboxynaminolimidazole;  
10 and metalloproteinase inhibitors such as BB94.

#### **Diseases at the Cellular Level**

Diseases associated with increased cell survival or the inhibition of apoptosis that could be treated, prevented, and/or diagnosed by the polynucleotides or  
15 polypeptides and/or antagonists or agonists of the invention, include cancers (such as follicular lymphomas, carcinomas with p53 mutations, and hormone-dependent tumors, including, but not limited to colon cancer, cardiac tumors, pancreatic cancer, melanoma, retinoblastoma, glioblastoma, lung cancer, intestinal cancer, testicular cancer, stomach cancer, neuroblastoma, myxoma, myoma, lymphoma, endothelioma,  
20 osteoblastoma, osteoclastoma, osteosarcoma, chondrosarcoma, adenoma, breast cancer, prostate cancer, Kaposi's sarcoma and ovarian cancer); autoimmune diseases, disorders, and/or conditions (such as, multiple sclerosis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related glomerulonephritis  
25 and rheumatoid arthritis) and viral infections (such as herpes viruses, pox viruses and adenoviruses), inflammation, graft v. host disease, acute graft rejection, and chronic

graft rejection. In preferred embodiments, the polynucleotides or polypeptides, and/or agonists or antagonists of the invention are used to inhibit growth, progression, and/or metasis of cancers, in particular those listed above.

Additional diseases or conditions associated with increased cell survival that  
5 could be treated, prevented or diagnosed by the polynucleotides or polypeptides, or agonists or antagonists of the invention, include, but are not limited to, progression, and/or metastases of malignancies and related disorders such as leukemia (including acute leukemias (e.g., acute lymphocytic leukemia, acute myelocytic leukemia (including myeloblastic, promyelocytic, myelomonocytic, monocytic, and  
10 erythroleukemia)) and chronic leukemias (e.g., chronic myelocytic (granulocytic) leukemia and chronic lymphocytic leukemia)), polycythemia vera, lymphomas (e.g., Hodgkin's disease and non-Hodgkin's disease), multiple myeloma, Waldenstrom's macroglobulinemia, heavy chain disease, and solid tumors including, but not limited to, sarcomas and carcinomas such as fibrosarcoma, myxosarcoma, liposarcoma,  
15 chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma,  
20 papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma,  
25 craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, menangioma, melanoma, neuroblastoma, and retinoblastoma.

Diseases associated with increased apoptosis that could be treated, prevented, and/or diagnosed by the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, include AIDS; neurodegenerative diseases, disorders, and/or conditions (such as Alzheimer's disease, Parkinson's disease, Amyotrophic lateral sclerosis, Retinitis pigmentosa, Cerebellar degeneration and brain tumor or prior associated disease); autoimmune diseases, disorders, and/or conditions (such as, multiple sclerosis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related glomerulonephritis and rheumatoid arthritis) myelodysplastic syndromes (such as aplastic anemia), graft v. host disease, ischemic injury (such as that caused by myocardial infarction, stroke and reperfusion injury), liver injury (e.g., hepatitis related liver injury, ischemia/reperfusion injury, cholestasis (bile duct injury) and liver cancer); toxin-induced liver disease (such as that caused by alcohol), septic shock, cachexia and anorexia.

15

#### **Wound Healing and Epithelial Cell Proliferation**

In accordance with yet a further aspect of the present invention, there is provided a process for utilizing the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, for therapeutic purposes, for example, to stimulate epithelial cell proliferation and basal keratinocytes for the purpose of wound healing, and to stimulate hair follicle production and healing of dermal wounds. Polynucleotides or polypeptides, as well as agonists or antagonists of the invention, may be clinically useful in stimulating wound healing including surgical wounds, excisional wounds, deep wounds involving damage of the dermis and epidermis, eye tissue wounds, dental tissue wounds, oral cavity wounds, diabetic ulcers, dermal ulcers, cubitus ulcers, arterial ulcers, venous stasis ulcers, burns resulting from heat

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25



exposure or chemicals, and other abnormal wound healing conditions such as uremia, malnutrition, vitamin deficiencies and complications associated with systemic treatment with steroids, radiation therapy and antineoplastic drugs and antimetabolites. Polynucleotides or polypeptides, and/or agonists or antagonists of  
5 the invention, could be used to promote dermal reestablishment subsequent to dermal loss

The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used to increase the adherence of skin grafts to a wound bed and to stimulate re-epithelialization from the wound bed. The following are a non-  
10 exhaustive list of grafts that polynucleotides or polypeptides, agonists or antagonists of the invention, could be used to increase adherence to a wound bed: autografts, artificial skin, allografts, autodermic graft, autoepidermic grafts, avacular grafts, Blair-Brown grafts, bone graft, brephoplastic grafts, cutis graft, delayed graft, dermic graft, epidermic graft, fascia graft, full thickness graft, heterologous graft, xenograft,  
15 homologous graft, hyperplastic graft, lamellar graft, mesh graft, mucosal graft, Ollier-Thiersch graft, omentopial graft, patch graft, pedicle graft, penetrating graft, split skin graft, thick split graft. The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, can be used to promote skin strength and to improve the appearance of aged skin.

20 It is believed that the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, will also produce changes in hepatocyte proliferation, and epithelial cell proliferation in the lung, breast, pancreas, stomach, small intestine, and large intestine. The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could promote proliferation of epithelial cells such as  
25 sebocytes, hair follicles, hepatocytes, type II pneumocytes, mucin-producing goblet cells, and other epithelial cells and their progenitors contained within the skin, lung,

liver, and gastrointestinal tract. The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, may promote proliferation of endothelial cells, keratinocytes, and basal keratinocytes.

The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could also be used to reduce the side effects of gut toxicity that result from radiation, chemotherapy treatments or viral infections. The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, may have a cytoprotective effect on the small intestine mucosa. The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, may also stimulate healing of mucositis (mouth ulcers) that result from chemotherapy and viral infections.

The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could further be used in full regeneration of skin in full and partial thickness skin defects, including burns, (i.e., repopulation of hair follicles, sweat glands, and sebaceous glands), treatment of other skin defects such as psoriasis. The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used to treat epidermolysis bullosa, a defect in adherence of the epidermis to the underlying dermis which results in frequent, open and painful blisters by accelerating reepithelialization of these lesions. The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could also be used to treat gastric and duodenal ulcers and help heal by scar formation of the mucosal lining and regeneration of glandular mucosa and duodenal mucosal lining more rapidly. Inflammatory bowel diseases, such as Crohn's disease and ulcerative colitis, are diseases which result in destruction of the mucosal surface of the small or large intestine, respectively. Thus, the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used to promote the resurfacing of the mucosal

surface to aid more rapid healing and to prevent progression of inflammatory bowel disease. Treatment with the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, is expected to have a significant effect on the production of mucus throughout the gastrointestinal tract and could be used to protect the intestinal mucosa from injurious substances that are ingested or following surgery. The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used to treat diseases associate with the under expression of the polynucleotides of the invention.

Moreover, the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used to prevent and heal damage to the lungs due to various pathological states. A growth factor such as the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, which could stimulate proliferation and differentiation and promote the repair of alveoli and brochiolar epithelium to prevent or treat acute or chronic lung damage. For example, emphysema, which results in the progressive loss of aveoli, and inhalation injuries, i.e., resulting from smoke inhalation and burns, that cause necrosis of the bronchiolar epithelium and alveoli could be effectively treated, prevented, and/or diagnosed using the polynucleotides or polypeptides, and/or agonists or antagonists of the invention. Also, the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used to stimulate the proliferation of and differentiation of type II pneumocytes, which may help treat or prevent disease such as hyaline membrane diseases, such as infant respiratory distress syndrome and bronchopulmonary displasia, in premature infants.

The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could stimulate the proliferation and differentiation of hepatocytes and, thus, could be used to alleviate or treat liver diseases and pathologies such as

fulminant liver failure caused by cirrhosis, liver damage caused by viral hepatitis and toxic substances (i.e., acetaminophen, carbon tetrachloride and other hepatotoxins known in the art).

In addition, the polynucleotides or polypeptides, and/or agonists or antagonists  
5 of the invention, could be used to treat or prevent the onset of diabetes mellitus. In patients with newly diagnosed Types I and II diabetes, where some islet cell function remains, the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used to maintain the islet function so as to alleviate, delay or prevent permanent manifestation of the disease. Also, the polynucleotides or  
10 polypeptides, and/or agonists or antagonists of the invention, could be used as an auxiliary in islet cell transplantation to improve or promote islet cell function.

### Neurological Diseases

15 Nervous system diseases, disorders, and/or conditions, which can be treated, prevented, and/or diagnosed with the compositions of the invention (e.g., polypeptides, polynucleotides, and/or agonists or antagonists), include, but are not limited to, nervous system injuries, and diseases, disorders, and/or conditions which result in either a disconnection of axons, a diminution or degeneration of neurons, or  
20 demyelination. Nervous system lesions which may be treated, prevented, and/or diagnosed in a patient (including human and non-human mammalian patients) according to the invention, include but are not limited to, the following lesions of either the central (including spinal cord, brain) or peripheral nervous systems: (1) ischemic lesions, in which a lack of oxygen in a portion of the nervous system results  
25 in neuronal injury or death, including cerebral infarction or ischemia, or spinal cord infarction or ischemia; (2) traumatic lesions, including lesions caused by physical

injury or associated with surgery, for example, lesions which sever a portion of the nervous system, or compression injuries; (3) malignant lesions, in which a portion of the nervous system is destroyed or injured by malignant tissue which is either a nervous system associated malignancy or a malignancy derived from non-nervous system tissue; (4) infectious lesions, in which a portion of the nervous system is destroyed or injured as a result of infection, for example, by an abscess or associated with infection by human immunodeficiency virus, herpes zoster, or herpes simplex virus or with Lyme disease, tuberculosis, syphilis; (5) degenerative lesions, in which a portion of the nervous system is destroyed or injured as a result of a degenerative process including but not limited to degeneration associated with Parkinson's disease, Alzheimer's disease, Huntington's chorea, or amyotrophic lateral sclerosis (ALS); (6) lesions associated with nutritional diseases, disorders, and/or conditions, in which a portion of the nervous system is destroyed or injured by a nutritional disorder or disorder of metabolism including but not limited to, vitamin B12 deficiency, folic acid deficiency, Wernicke disease, tobacco-alcohol amblyopia, Marchiafava-Bignami disease (primary degeneration of the corpus callosum), and alcoholic cerebellar degeneration; (7) neurological lesions associated with systemic diseases including, but not limited to, diabetes (diabetic neuropathy, Bell's palsy), systemic lupus erythematosus, carcinoma, or sarcoidosis; (8) lesions caused by toxic substances including alcohol, lead, or particular neurotoxins; and (9) demyelinated lesions in which a portion of the nervous system is destroyed or injured by a demyelinating disease including, but not limited to, multiple sclerosis, human immunodeficiency virus-associated myelopathy, transverse myelopathy or various etiologies, progressive multifocal leukoencephalopathy, and central pontine myelinolysis.

In a preferred embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the invention are used to protect neural cells from the damaging effects

of cerebral hypoxia. According to this embodiment, the compositions of the invention are used to treat, prevent, and/or diagnose neural cell injury associated with cerebral hypoxia. In one aspect of this embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the invention are used to treat, prevent, and/or diagnose neural cell injury associated with cerebral ischemia. In another aspect of this embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the invention are used to treat, prevent, and/or diagnose neural cell injury associated with cerebral infarction. In another aspect of this embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the invention are used to treat, prevent, and/or diagnose or prevent neural cell injury associated with a stroke. In a further aspect of this embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the invention are used to treat, prevent, and/or diagnose neural cell injury associated with a heart attack.

The compositions of the invention which are useful for treating or preventing a nervous system disorder may be selected by testing for biological activity in promoting the survival or differentiation of neurons. For example, and not by way of limitation, compositions of the invention which elicit any of the following effects may be useful according to the invention: (1) increased survival time of neurons in culture; (2) increased sprouting of neurons in culture or *in vivo*; (3) increased production of a neuron-associated molecule in culture or *in vivo*, *e.g.*, choline acetyltransferase or acetylcholinesterase with respect to motor neurons; or (4) decreased symptoms of neuron dysfunction *in vivo*. Such effects may be measured by any method known in the art. In preferred, non-limiting embodiments, increased survival of neurons may routinely be measured using a method set forth herein or otherwise known in the art, such as, for example, the method set forth in Arakawa et al. (J. Neurosci. 10:3507-3515 (1990)); increased sprouting of neurons may be detected by methods

known in the art, such as, for example, the methods set forth in Pestronk et al. (Exp. Neurol. 70:65-82 (1980)) or Brown et al. (Ann. Rev. Neurosci. 4:17-42 (1981)); increased production of neuron-associated molecules may be measured by bioassay, enzymatic assay, antibody binding, Northern blot assay, etc., using techniques known in the art and depending on the molecule to be measured; and motor neuron dysfunction may be measured by assessing the physical manifestation of motor neuron disorder, e.g., weakness, motor neuron conduction velocity, or functional disability.

In specific embodiments, motor neuron diseases, disorders, and/or conditions that may be treated, prevented, and/or diagnosed according to the invention include, but are not limited to, diseases, disorders, and/or conditions such as infarction, infection, exposure to toxin, trauma, surgical damage, degenerative disease or malignancy that may affect motor neurons as well as other components of the nervous system, as well as diseases, disorders, and/or conditions that selectively affect neurons such as amyotrophic lateral sclerosis, and including, but not limited to, progressive spinal muscular atrophy, progressive bulbar palsy, primary lateral sclerosis, infantile and juvenile muscular atrophy, progressive bulbar paralysis of childhood (Fazio-Londe syndrome), poliomyelitis and the post polio syndrome, and Hereditary Motorsensory Neuropathy (Charcot-Marie-Tooth Disease).

### **Infectious Disease**

A polypeptide or polynucleotide and/or agonist or antagonist of the present invention can be used to treat, prevent, and/or diagnose infectious agents. For example, by increasing the immune response, particularly increasing the proliferation and differentiation of B and/or T cells, infectious diseases may be treated, prevented, and/or diagnosed. The immune response may be increased by either enhancing an

existing immune response, or by initiating a new immune response. Alternatively, polypeptide or polynucleotide and/or agonist or antagonist of the present invention may also directly inhibit the infectious agent, without necessarily eliciting an immune response.

- 5           Viruses are one example of an infectious agent that can cause disease or symptoms that can be treated, prevented, and/or diagnosed by a polynucleotide or polypeptide and/or agonist or antagonist of the present invention. Examples of viruses, include, but are not limited to Examples of viruses, include, but are not limited to the following DNA and RNA viruses and viral families: Arbovirus,
- 10   Adenoviridae, Arenaviridae, Arterivirus, Birnaviridae, Bunyaviridae, Caliciviridae, Circoviridae, Coronaviridae, Dengue, EBV, HIV, Flaviviridae, Hepadnaviridae (Hepatitis), Herpesviridae (such as, Cytomegalovirus, Herpes Simplex, Herpes Zoster), Mononegavirus (e.g., Paramyxoviridae, Morbillivirus, Rhabdoviridae), Orthomyxoviridae (e.g., Influenza A, Influenza B, and parainfluenza), Papiloma
- 15   virus, Papovaviridae, Parvoviridae, Picornaviridae, Poxviridae (such as Smallpox or Vaccinia), Reoviridae (e.g., Rotavirus), Retroviridae (HTLV-I, HTLV-II, Lentivirus), and Togaviridae (e.g., Rubivirus). Viruses falling within these families can cause a variety of diseases or symptoms, including, but not limited to: arthritis, bronchiollitis, respiratory syncytial virus, encephalitis, eye infections (e.g., conjunctivitis, keratitis),
- 20   chronic fatigue syndrome, hepatitis (A, B, C, E, Chronic Active, Delta), Japanese B encephalitis, Junin, Chikungunya, Rift Valley fever, yellow fever, meningitis, opportunistic infections (e.g., AIDS), pneumonia, Burkitt's Lymphoma, chickenpox, hemorrhagic fever, Measles, Mumps, Parainfluenza, Rabies, the common cold, Polio, leukemia, Rubella, sexually transmitted diseases, skin diseases (e.g., Kaposi's, warts),
- 25   and viremia. polynucleotides or polypeptides, or agonists or antagonists of the invention, can be used to treat, prevent, and/or diagnose any of these symptoms or



diseases. In specific embodiments, polynucleotides, polypeptides, or agonists or antagonists of the invention are used to treat, prevent, and/or diagnose: meningitis, Dengue, EBV, and/or hepatitis (e.g., hepatitis B). In an additional specific embodiment polynucleotides, polypeptides, or agonists or antagonists of the invention are used to treat patients nonresponsive to one or more other commercially available hepatitis vaccines. In a further specific embodiment polynucleotides, polypeptides, or agonists or antagonists of the invention are used to treat, prevent, and/or diagnose AIDS.

Similarly, bacterial or fungal agents that can cause disease or symptoms and that can be treated, prevented, and/or diagnosed by a polynucleotide or polypeptide and/or agonist or antagonist of the present invention include, but not limited to, include, but not limited to, the following Gram-Negative and Gram-positive bacteria and bacterial families and fungi: Actinomycetales (e.g., *Corynebacterium*, *Mycobacterium*, *Nocardia*), *Cryptococcus neoformans*, *Aspergillosis*, *Bacillaceae* (e.g., *Anthrax*, *Clostridium*), *Bacteroidaceae*, *Blastomycosis*, *Bordetella*, *Borrelia* (e.g., *Borrelia burgdorferi*), *Brucellosis*, *Candidiasis*, *Campylobacter*, *Coccidioidomycosis*, *Cryptococcosis*, *Dermatocycoses*, *E. coli* (e.g., *Enterotoxigenic E. coli* and *Enterohemorrhagic E. coli*), *Enterobacteriaceae* (*Klebsiella*, *Salmonella* (e.g., *Salmonella typhi*, and *Salmonella paratyphi*), *Serratia*, *Yersinia*), *Erysipelothrix*, *Helicobacter*, *Legionellosis*, *Leptospirosis*, *Listeria*, *Mycoplasmatales*, *Mycobacterium leprae*, *Vibrio cholerae*, *Neisseriaceae* (e.g., *Acinetobacter*, *Gonorrhea*, *Menigococcal*), *Meisseria meningitidis*, *Pasteurellacea Infections* (e.g., *Actinobacillus*, *Heamophilus* (e.g., *Heamophilus influenza type B*), *Pasteurella*), *Pseudomonas*, *Rickettsiaceae*, *Chlamydiaceae*, *Syphilis*, *Shigella spp.*, *Staphylococcal*, *Meningiococcal*, *Pneumococcal* and *Streptococcal* (e.g., *Streptococcus pneumoniae* and *Group B Streptococcus*). These bacterial or fungal

families can cause the following diseases or symptoms, including, but not limited to: bacteremia, endocarditis, eye infections (conjunctivitis, tuberculosis, uveitis), gingivitis, opportunistic infections (e.g., AIDS related infections), paronychia, prosthesis-related infections, Reiter's Disease, respiratory tract infections, such as

5 Whooping Cough or Empyema, sepsis, Lyme Disease, Cat-Scratch Disease, Dysentery, Paratyphoid Fever, food poisoning, Typhoid, pneumonia, Gonorrhea, meningitis (e.g., meningitis types A and B), Chlamydia, Syphilis, Diphtheria, Leprosy, Paratuberculosis, Tuberculosis, Lupus, Botulism, gangrene, tetanus, impetigo,

10 Rheumatic Fever, Scarlet Fever, sexually transmitted diseases, skin diseases (e.g., cellulitis, dermatocycoses), toxemia, urinary tract infections, wound infections.

Polynucleotides or polypeptides, agonists or antagonists of the invention, can be used to treat, prevent, and/or diagnose any of these symptoms or diseases. In specific embodiments, polynucleotides, polypeptides, agonists or antagonists of the invention are used to treat, prevent, and/or diagnose: tetanus, Diphtheria, botulism, and/or

15 meningitis type B.

Moreover, parasitic agents causing disease or symptoms that can be treated, prevented, and/or diagnosed by a polynucleotide or polypeptide and/or agonist or antagonist of the present invention include, but not limited to, the following families or class: Amebiasis, Babesiosis, Coccidiosis, Cryptosporidiosis, Dientamoebiasis,

20 Dourine, Ectoparasitic, Giardiasis, Helminthiasis, Leishmaniasis, Theileriasis, Toxoplasmosis, Trypanosomiasis, and Trichomonas and Sporozoans (e.g., Plasmodium virax, Plasmodium falciparum, Plasmodium malariae and Plasmodium ovale). These parasites can cause a variety of diseases or symptoms, including, but not limited to: Scabies, Trombiculiasis, eye infections, intestinal disease (e.g.,

25 dysentery, giardiasis), liver disease, lung disease, opportunistic infections (e.g., AIDS related), malaria, pregnancy complications, and toxoplasmosis. polynucleotides or

polypeptides, or agonists or antagonists of the invention, can be used to treat, prevent, and/or diagnose any of these symptoms or diseases. In specific embodiments, polynucleotides, polypeptides, or agonists or antagonists of the invention are used to treat, prevent, and/or diagnose malaria.

- 5            Preferably, treatment or prevention using a polypeptide or polynucleotide and/or agonist or antagonist of the present invention could either be by administering an effective amount of a polypeptide to the patient, or by removing cells from the patient, supplying the cells with a polynucleotide of the present invention, and returning the engineered cells to the patient (ex vivo therapy). Moreover, the
- 10           polypeptide or polynucleotide of the present invention can be used as an antigen in a vaccine to raise an immune response against infectious disease.

### **Regeneration**

- A polynucleotide or polypeptide and/or agonist or antagonist of the present
- 15           invention can be used to differentiate, proliferate, and attract cells, leading to the regeneration of tissues. (See, Science 276:59-87 (1997).) The regeneration of tissues could be used to repair, replace, or protect tissue damaged by congenital defects, trauma (wounds, burns, incisions, or ulcers), age, disease (e.g. osteoporosis, osteoarthritis, periodontal disease, liver failure), surgery, including cosmetic plastic
- 20           surgery, fibrosis, reperfusion injury, or systemic cytokine damage.

- Tissues that could be regenerated using the present invention include organs (e.g., pancreas, liver, intestine, kidney, skin, endothelium), muscle (smooth, skeletal or cardiac), vasculature (including vascular and lymphatics), nervous, hematopoietic, and skeletal (bone, cartilage, tendon, and ligament) tissue. Preferably, regeneration
- 25           occurs without or decreased scarring. Regeneration also may include angiogenesis.

Moreover, a polynucleotide or polypeptide and/or agonist or antagonist of the present invention may increase regeneration of tissues difficult to heal. For example, increased tendon/ligament regeneration would quicken recovery time after damage.

A polynucleotide or polypeptide and/or agonist or antagonist of the present invention  
5 could also be used prophylactically in an effort to avoid damage. Specific diseases that could be treated, prevented, and/or diagnosed include of tendinitis, carpal tunnel syndrome, and other tendon or ligament defects. A further example of tissue regeneration of non-healing wounds includes pressure ulcers, ulcers associated with vascular insufficiency, surgical, and traumatic wounds.

10 Similarly, nerve and brain tissue could also be regenerated by using a polynucleotide or polypeptide and/or agonist or antagonist of the present invention to proliferate and differentiate nerve cells. Diseases that could be treated, prevented, and/or diagnosed using this method include central and peripheral nervous system diseases, neuropathies, or mechanical and traumatic diseases, disorders, and/or  
15 conditions (e.g., spinal cord disorders, head trauma, cerebrovascular disease, and stroke). Specifically, diseases associated with peripheral nerve injuries, peripheral neuropathy (e.g., resulting from chemotherapy or other medical therapies), localized neuropathies, and central nervous system diseases (e.g., Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and Shy-  
20 Drager syndrome), could all be treated, prevented, and/or diagnosed using the polynucleotide or polypeptide and/or agonist or antagonist of the present invention.

### Chemotaxis

A polynucleotide or polypeptide and/or agonist or antagonist of the present  
25 invention may have chemotaxis activity. A chemotaxic molecule attracts or mobilizes cells (e.g., monocytes, fibroblasts, neutrophils, T-cells, mast cells, eosinophils,

epithelial and/or endothelial cells) to a particular site in the body, such as inflammation, infection, or site of hyperproliferation. The mobilized cells can then fight off and/or heal the particular trauma or abnormality.

A polynucleotide or polypeptide and/or agonist or antagonist of the present invention may increase chemotactic activity of particular cells. These chemotactic molecules can then be used to treat, prevent, and/or diagnose inflammation, infection, hyperproliferative diseases, disorders, and/or conditions, or any immune system disorder by increasing the number of cells targeted to a particular location in the body. For example, chemotactic molecules can be used to treat, prevent, and/or diagnose wounds and other trauma to tissues by attracting immune cells to the injured location. Chemotactic molecules of the present invention can also attract fibroblasts, which can be used to treat, prevent, and/or diagnose wounds.

It is also contemplated that a polynucleotide or polypeptide and/or agonist or antagonist of the present invention may inhibit chemotactic activity. These molecules could also be used to treat, prevent, and/or diagnose diseases, disorders, and/or conditions. Thus, a polynucleotide or polypeptide and/or agonist or antagonist of the present invention could be used as an inhibitor of chemotaxis.

## 20 **Binding Activity**

A polypeptide of the present invention may be used to screen for molecules that bind to the polypeptide or for molecules to which the polypeptide binds. The binding of the polypeptide and the molecule may activate (agonist), increase, inhibit (antagonist), or decrease activity of the polypeptide or the molecule bound. Examples of such molecules include antibodies, oligonucleotides, proteins (e.g., receptors), or small molecules.

Preferably, the molecule is closely related to the natural ligand of the polypeptide, e.g., a fragment of the ligand, or a natural substrate, a ligand, a structural or functional mimetic. (See, Coligan et al., Current Protocols in Immunology 1(2):Chapter 5 (1991).) Similarly, the molecule can be closely related to the natural  
5 receptor to which the polypeptide binds, or at least, a fragment of the receptor capable of being bound by the polypeptide (e.g., active site). In either case, the molecule can be rationally designed using known techniques.

Preferably, the screening for these molecules involves producing appropriate cells which express the polypeptide, either as a secreted protein or on the cell  
10 membrane. Preferred cells include cells from mammals, yeast, *Drosophila*, or *E. coli*. Cells expressing the polypeptide (or cell membrane containing the expressed polypeptide) are then preferably contacted with a test compound potentially containing the molecule to observe binding, stimulation, or inhibition of activity of either the polypeptide or the molecule.

15 The assay may simply test binding of a candidate compound to the polypeptide, wherein binding is detected by a label, or in an assay involving competition with a labeled competitor. Further, the assay may test whether the candidate compound results in a signal generated by binding to the polypeptide.

Alternatively, the assay can be carried out using cell-free preparations,  
20 polypeptide/molecule affixed to a solid support, chemical libraries, or natural product mixtures. The assay may also simply comprise the steps of mixing a candidate compound with a solution containing a polypeptide, measuring polypeptide/molecule activity or binding, and comparing the polypeptide/molecule activity or binding to a standard.

25 Preferably, an ELISA assay can measure polypeptide level or activity in a sample (e.g., biological sample) using a monoclonal or polyclonal antibody. The

antibody can measure polypeptide level or activity by either binding, directly or indirectly, to the polypeptide or by competing with the polypeptide for a substrate.

Additionally, the receptor to which a polypeptide of the invention binds can be identified by numerous methods known to those of skill in the art, for example, ligand panning and FACS sorting (Coligan, et al., Current Protocols in Immun., 1(2), Chapter 5, (1991)). For example, expression cloning is employed wherein polyadenylated RNA is prepared from a cell responsive to the polypeptides, for example, NIH3T3 cells which are known to contain multiple receptors for the FGF family proteins, and SC-3 cells, and a cDNA library created from this RNA is divided into pools and used to transfect COS cells or other cells that are not responsive to the polypeptides. Transfected cells which are grown on glass slides are exposed to the polypeptide of the present invention, after they have been labelled. The polypeptides can be labeled by a variety of means including iodination or inclusion of a recognition site for a site-specific protein kinase.

Following fixation and incubation, the slides are subjected to autoradiographic analysis. Positive pools are identified and sub-pools are prepared and re-transfected using an iterative sub-pooling and re-screening process, eventually yielding a single clones that encodes the putative receptor.

As an alternative approach for receptor identification, the labeled polypeptides can be photoaffinity linked with cell membrane or extract preparations that express the receptor molecule. Cross-linked material is resolved by PAGE analysis and exposed to X-ray film. The labeled complex containing the receptors of the polypeptides can be excised, resolved into peptide fragments, and subjected to protein microsequencing. The amino acid sequence obtained from microsequencing would be used to design a set of degenerate oligonucleotide probes to screen a cDNA library to identify the genes encoding the putative receptors.

Moreover, the techniques of gene-shuffling, motif-shuffling, exon-shuffling, and/or codon-shuffling (collectively referred to as "DNA shuffling") may be employed to modulate the activities of polypeptides of the invention thereby effectively generating agonists and antagonists of polypeptides of the invention. See generally, U.S. Patent Nos. 5,605,793, 5,811,238, 5,830,721, 5,834,252, and 5,837,458, and Patten, P. A., et al., *Curr. Opinion Biotechnol.* 8:724-33 (1997); Harayama, S. *Trends Biotechnol.* 16(2):76-82 (1998); Hansson, L. O., et al., *J. Mol. Biol.* 287:265-76 (1999); and Lorenzo, M. M. and Blasco, R. *Biotechniques* 24(2):308-13 (1998) (each of these patents and publications are hereby incorporated by reference). In one embodiment, alteration of polynucleotides and corresponding polypeptides of the invention may be achieved by DNA shuffling. DNA shuffling involves the assembly of two or more DNA segments into a desired polynucleotide sequence of the invention molecule by homologous, or site-specific, recombination. In another embodiment, polynucleotides and corresponding polypeptides of the invention may be altered by being subjected to random mutagenesis by error-prone PCR, random nucleotide insertion or other methods prior to recombination. In another embodiment, one or more components, motifs, sections, parts, domains, fragments, etc., of the polypeptides of the invention may be recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous molecules. In preferred embodiments, the heterologous molecules are family members. In further preferred embodiments, the heterologous molecule is a growth factor such as, for example, platelet-derived growth factor (PDGF), insulin-like growth factor (IGF-I), transforming growth factor (TGF)-alpha, epidermal growth factor (EGF), fibroblast growth factor (FGF), TGF-beta, bone morphogenetic protein (BMP)-2, BMP-4, BMP-5, BMP-6, BMP-7, activins A and B, decapentaplegic(dpp), 60A, OP-2, dorsalin, growth differentiation factors (GDFs),



nodal, MIS, inhibin-alpha, TGF-beta1, TGF-beta2, TGF-beta3, TGF-beta5, and glial-derived neurotrophic factor (GDNF).

Other preferred fragments are biologically active fragments of the polypeptides of the invention. Biologically active fragments are those exhibiting  
5 activity similar, but not necessarily identical, to an activity of the polypeptide. The biological activity of the fragments may include an improved desired activity, or a decreased undesirable activity.

Additionally, this invention provides a method of screening compounds to identify those which modulate the action of the polypeptide of the present invention.  
10 An example of such an assay comprises combining a mammalian fibroblast cell, a the polypeptide of the present invention, the compound to be screened and 3[H] thymidine under cell culture conditions where the fibroblast cell would normally proliferate. A control assay may be performed in the absence of the compound to be screened and compared to the amount of fibroblast proliferation in the presence of the  
15 compound to determine if the compound stimulates proliferation by determining the uptake of 3[H] thymidine in each case. The amount of fibroblast cell proliferation is measured by liquid scintillation chromatography which measures the incorporation of 3[H] thymidine. Both agonist and antagonist compounds may be identified by this procedure.

20 In another method, a mammalian cell or membrane preparation expressing a receptor for a polypeptide of the present invention is incubated with a labeled polypeptide of the present invention in the presence of the compound. The ability of the compound to enhance or block this interaction could then be measured. Alternatively, the response of a known second messenger system following  
25 interaction of a compound to be screened and the receptor is measured and the ability of the compound to bind to the receptor and elicit a second messenger response is

measured to determine if the compound is a potential agonist or antagonist. Such second messenger systems include but are not limited to, cAMP guanylate cyclase, ion channels or phosphoinositide hydrolysis.

All of these above assays can be used as diagnostic or prognostic markers.

- 5 The molecules discovered using these assays can be used to treat, prevent, and/or diagnose disease or to bring about a particular result in a patient (e.g., blood vessel growth) by activating or inhibiting the polypeptide/molecule. Moreover, the assays can discover agents which may inhibit or enhance the production of the polypeptides of the invention from suitably manipulated cells or tissues. Therefore, the invention
- 10 includes a method of identifying compounds which bind to the polypeptides of the invention comprising the steps of: (a) incubating a candidate binding compound with the polypeptide; and (b) determining if binding has occurred. Moreover, the invention includes a method of identifying agonists/antagonists comprising the steps of: (a) incubating a candidate compound with the polypeptide, (b) assaying a
- 15 biological activity, and (b) determining if a biological activity of the polypeptide has been altered.

- Also, one could identify molecules bind a polypeptide of the invention experimentally by using the beta-pleated sheet regions contained in the polypeptide sequence of the protein. Accordingly, specific embodiments of the invention are
- 20 directed to polynucleotides encoding polypeptides which comprise, or alternatively consist of, the amino acid sequence of each beta pleated sheet regions in a disclosed polypeptide sequence. Additional embodiments of the invention are directed to polynucleotides encoding polypeptides which comprise, or alternatively consist of, any combination or all of contained in the polypeptide sequences of the invention.
- 25 Additional preferred embodiments of the invention are directed to polypeptides which comprise, or alternatively consist of, the amino acid sequence of each of the beta

pleated sheet regions in one of the polypeptide sequences of the invention. Additional embodiments of the invention are directed to polypeptides which comprise, or alternatively consist of, any combination or all of the beta pleated sheet regions in one of the polypeptide sequences of the invention.

5

### **Targeted Delivery**

In another embodiment, the invention provides a method of delivering compositions to targeted cells expressing a receptor for a polypeptide of the invention, or cells expressing a cell bound form of a polypeptide of the invention.

10

As discussed herein, polypeptides or antibodies of the invention may be associated with heterologous polypeptides, heterologous nucleic acids, toxins, or prodrugs via hydrophobic, hydrophilic, ionic and/or covalent interactions. In one embodiment, the invention provides a method for the specific delivery of compositions of the invention to cells by administering polypeptides of the invention (including antibodies) that are associated with heterologous polypeptides or nucleic acids. In one example, the invention provides a method for delivering a therapeutic protein into the targeted cell. In another example, the invention provides a method for delivering a single stranded nucleic acid (e.g., antisense or ribozymes) or double stranded nucleic acid (e.g., DNA that can integrate into the cell's genome or replicate episomally and that can be transcribed) into the targeted cell.

15

20

In another embodiment, the invention provides a method for the specific destruction of cells (e.g., the destruction of tumor cells) by administering polypeptides of the invention (e.g., polypeptides of the invention or antibodies of the invention) in association with toxins or cytotoxic prodrugs.

25

By "toxin" is meant compounds that bind and activate endogenous cytotoxic effector systems, radioisotopes, holotoxins, modified toxins, catalytic subunits of

toxins, or any molecules or enzymes not normally present in or on the surface of a cell that under defined conditions cause the cell's death. Toxins that may be used according to the methods of the invention include, but are not limited to, radioisotopes known in the art, compounds such as, for example, antibodies (or complement fixing  
5 containing portions thereof) that bind an inherent or induced endogenous cytotoxic effector system, thymidine kinase, endonuclease, RNase, alpha toxin, ricin, abrin, *Pseudomonas* exotoxin A, diphtheria toxin, saporin, momordin, gelonin, pokeweed antiviral protein, alpha-sarcin and cholera toxin. By "cytotoxic prodrug" is meant a non-toxic compound that is converted by an enzyme, normally present in the cell, into  
10 a cytotoxic compound. Cytotoxic prodrugs that may be used according to the methods of the invention include, but are not limited to, glutamyl derivatives of benzoic acid mustard alkylating agent, phosphate derivatives of etoposide or mitomycin C, cytosine arabinoside, daunorubisin, and phenoxyacetamide derivatives of doxorubicin.

15

#### Drug Screening

Further contemplated is the use of the polypeptides of the present invention, or the polynucleotides encoding these polypeptides, to screen for molecules which modify the activities of the polypeptides of the present invention. Such a method  
20 would include contacting the polypeptide of the present invention with a selected compound(s) suspected of having antagonist or agonist activity, and assaying the activity of these polypeptides following binding.

This invention is particularly useful for screening therapeutic compounds by using the polypeptides of the present invention, or binding fragments thereof, in any  
25 of a variety of drug screening techniques. The polypeptide or fragment employed in such a test may be affixed to a solid support, expressed on a cell surface, free in

solution, or located intracellularly. One method of drug screening utilizes eukaryotic or prokaryotic host cells which are stably transformed with recombinant nucleic acids expressing the polypeptide or fragment. Drugs are screened against such transformed cells in competitive binding assays. One may measure, for example, the formulation  
5 of complexes between the agent being tested and a polypeptide of the present invention.

Thus, the present invention provides methods of screening for drugs or any other agents which affect activities mediated by the polypeptides of the present invention. These methods comprise contacting such an agent with a polypeptide of the  
10 present invention or a fragment thereof and assaying for the presence of a complex between the agent and the polypeptide or a fragment thereof, by methods well known in the art. In such a competitive binding assay, the agents to screen are typically labeled. Following incubation, free agent is separated from that present in bound form, and the amount of free or uncomplexed label is a measure of the ability of a  
15 particular agent to bind to the polypeptides of the present invention.

Another technique for drug screening provides high throughput screening for compounds having suitable binding affinity to the polypeptides of the present invention, and is described in great detail in European Patent Application 84/03564, published on September 13, 1984, which is incorporated herein by reference herein.  
20 Briefly stated, large numbers of different small peptide test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. The peptide test compounds are reacted with polypeptides of the present invention and washed. Bound polypeptides are then detected by methods well known in the art. Purified polypeptides are coated directly onto plates for use in the aforementioned  
25 drug screening techniques. In addition, non-neutralizing antibodies may be used to capture the peptide and immobilize it on the solid support.

This invention also contemplates the use of competitive drug screening assays in which neutralizing antibodies capable of binding polypeptides of the present invention specifically compete with a test compound for binding to the polypeptides or fragments thereof. In this manner, the antibodies are used to detect the presence of  
5 any peptide which shares one or more antigenic epitopes with a polypeptide of the invention.

#### **Antisense And Ribozyme (Antagonists)**

In specific embodiments, antagonists according to the present invention are  
10 nucleic acids corresponding to the sequences contained in SEQ ID NO:X, or the complementary strand thereof, and/or to nucleotide sequences contained a deposited clone. In one embodiment, antisense sequence is generated internally by the organism, in another embodiment, the antisense sequence is separately administered (see, for example, O'Connor, Neurochem., 56:560 (1991). Oligodeoxynucleotides as  
15 Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Antisense technology can be used to control gene expression through antisense DNA or RNA, or through triple-helix formation. Antisense techniques are discussed for example, in Okano, Neurochem., 56:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Triple helix  
20 formation is discussed in, for instance, Lee et al., Nucleic Acids Research, 6:3073 (1979); Cooney et al., Science, 241:456 (1988); and Dervan et al., Science, 251:1300 (1991). The methods are based on binding of a polynucleotide to a complementary DNA or RNA.

For example, the use of c-myc and c-myb antisense RNA constructs to inhibit  
25 the growth of the non-lymphocytic leukemia cell line HL-60 and other cell lines was previously described. (Wickstrom et al. (1988); Anfossi et al. (1989)). These

experiments were performed in vitro by incubating cells with the oligoribonucleotide. A similar procedure for in vivo use is described in WO 91/15580. Briefly, a pair of oligonucleotides for a given antisense RNA is produced as follows: A sequence complimentary to the first 15 bases of the open reading frame is flanked by an EcoRI  
5 site on the 5' end and a HindIII site on the 3' end. Next, the pair of oligonucleotides is heated at 90°C for one minute and then annealed in 2X ligation buffer (20mM TRIS HCl pH 7.5, 10mM MgCl<sub>2</sub>, 10mM dithiothreitol (DTT) and 0.2 mM ATP) and then ligated to the EcoRI/Hind III site of the retroviral vector PMV7 (WO 91/15580).

For example, the 5' coding portion of a polynucleotide that encodes the mature  
10 polypeptide of the present invention may be used to design an antisense RNA oligonucleotide of from about 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription thereby preventing transcription and the production of the receptor. The antisense RNA oligonucleotide hybridizes to the mRNA in vivo and blocks translation of the  
15 mRNA molecule into receptor polypeptide.

In one embodiment, the antisense nucleic acid of the invention is produced intracellularly by transcription from an exogenous sequence. For example, a vector or a portion thereof, is transcribed, producing an antisense nucleic acid (RNA) of the invention. Such a vector would contain a sequence encoding the antisense nucleic  
20 acid of the invention. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired antisense RNA. Such vectors can be constructed by recombinant DNA technology methods standard in the art. Vectors can be plasmid, viral, or others known in the art, used for replication and expression in vertebrate cells. Expression of the sequence encoding a  
25 polypeptide of the invention, or fragments thereof, can be by any promoter known in the art to act in vertebrate, preferably human cells. Such promoters can be inducible

or constitutive. Such promoters include, but are not limited to, the SV40 early promoter region (Bernoist and Chambon, *Nature*, 29:304-310 (1981), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto et al., *Cell*, 22:787-797 (1980), the herpes thymidine promoter (Wagner et al., *Proc. Natl. Acad. Sci. U.S.A.*, 78:1441-1445 (1981), the regulatory sequences of the metallothionein gene (Brinster et al., *Nature*, 296:39-42 (1982)), etc.

The antisense nucleic acids of the invention comprise a sequence complementary to at least a portion of an RNA transcript of a gene of interest. However, absolute complementarity, although preferred, is not required. A sequence "complementary to at least a portion of an RNA," referred to herein, means a sequence having sufficient complementarity to be able to hybridize with the RNA, forming a stable duplex; in the case of double stranded antisense nucleic acids of the invention, a single strand of the duplex DNA may thus be tested, or triplex formation may be assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid. Generally, the larger the hybridizing nucleic acid, the more base mismatches with a RNA sequence of the invention it may contain and still form a stable duplex (or triplex as the case may be). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

Oligonucleotides that are complementary to the 5' end of the message, *e.g.*, the 5' untranslated sequence up to and including the AUG initiation codon, should work most efficiently at inhibiting translation. However, sequences complementary to the 3' untranslated sequences of mRNAs have been shown to be effective at inhibiting translation of mRNAs as well. See generally, Wagner, R., *Nature*, 372:333-335 (1994). Thus, oligonucleotides complementary to either the 5' - or 3' - non- translated, non-coding regions of a polynucleotide sequence of the invention



could be used in an antisense approach to inhibit translation of endogenous mRNA. Oligonucleotides complementary to the 5' untranslated region of the mRNA should include the complement of the AUG start codon. Antisense oligonucleotides complementary to mRNA coding regions are less efficient inhibitors of translation but could be used in accordance with the invention. Whether designed to hybridize to the 5' -, 3' - or coding region of mRNA, antisense nucleic acids should be at least six nucleotides in length, and are preferably oligonucleotides ranging from 6 to about 50 nucleotides in length. In specific aspects the oligonucleotide is at least 10 nucleotides, at least 17 nucleotides, at least 25 nucleotides or at least 50 nucleotides.

The polynucleotides of the invention can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone, for example, to improve stability of the molecule, hybridization, etc. The oligonucleotide may include other appended groups such as peptides (e.g., for targeting host cell receptors in vivo), or agents facilitating transport across the cell membrane (see, e.g., Letsinger et al., Proc. Natl. Acad. Sci. U.S.A. 86:6553-6556 (1989); Lemaitre et al., Proc. Natl. Acad. Sci., 84:648-652 (1987); PCT Publication NO: WO88/09810, published December 15, 1988) or the blood-brain barrier (see, e.g., PCT Publication NO: WO89/10134, published April 25, 1988), hybridization-triggered cleavage agents. (See, e.g., Krol et al., BioTechniques, 6:958-976 (1988)) or intercalating agents. (See, e.g., Zon, Pharm. Res., 5:539-549 (1988)). To this end, the oligonucleotide may be conjugated to another molecule, e.g., a peptide, hybridization triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent, etc.

The antisense oligonucleotide may comprise at least one modified base moiety which is selected from the group including, but not limited to, 5-fluorouracil,

5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 5 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, 10 queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine.

The antisense oligonucleotide may also comprise at least one modified sugar moiety selected from the group including, but not limited to, arabinose, 15 2-fluoroarabinose, xylulose, and hexose.

In yet another embodiment, the antisense oligonucleotide comprises at least one modified phosphate backbone selected from the group including, but not limited to, a phosphorothioate, a phosphorodithioate, a phosphoramidothioate, a 20 phosphoramidate, a phosphordiamidate, a methylphosphonate, an alkyl phosphotriester, and a formacetal or analog thereof.

In yet another embodiment, the antisense oligonucleotide is an a-anomeric oligonucleotide. An a-anomeric oligonucleotide forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual b-units, the strands 25 run parallel to each other (Gautier et al., Nucl. Acids Res., 15:6625-6641 (1987)). The oligonucleotide is a 2-O-methylribonucleotide (Inoue et al., Nucl. Acids Res.,

15:6131-6148 (1987)), or a chimeric RNA-DNA analogue (Inoue et al., FEBS Lett. 215:327-330 (1987)).

Polynucleotides of the invention may be synthesized by standard methods known in the art, e.g. by use of an automated DNA synthesizer (such as are  
5 commercially available from Biosearch, Applied Biosystems, etc.). As examples, phosphorothioate oligonucleotides may be synthesized by the method of Stein et al. (Nucl. Acids Res., 16:3209 (1988)), methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer supports (Sarin et al., Proc. Natl. Acad. Sci. U.S.A., 85:7448-7451 (1988)), etc.

10 While antisense nucleotides complementary to the coding region sequence of the invention could be used, those complementary to the transcribed untranslated region are most preferred.

Potential antagonists according to the invention also include catalytic RNA, or a ribozyme (See, e.g., PCT International Publication WO 90/11364, published  
15 October 4, 1990; Sarver et al, Science, 247:1222-1225 (1990). While ribozymes that cleave mRNA at site specific recognition sequences can be used to destroy mRNAs corresponding to the polynucleotides of the invention, the use of hammerhead ribozymes is preferred. Hammerhead ribozymes cleave mRNAs at locations dictated by flanking regions that form complementary base pairs with the target mRNA. The  
20 sole requirement is that the target mRNA have the following sequence of two bases: 5' -UG-3'. The construction and production of hammerhead ribozymes is well known in the art and is described more fully in Haseloff and Gerlach, Nature, 334:585-591 (1988). There are numerous potential hammerhead ribozyme cleavage sites within each nucleotide sequence disclosed in the sequence listing. Preferably,  
25 the ribozyme is engineered so that the cleavage recognition site is located near the 5' end of the mRNA corresponding to the polynucleotides of the invention; i.e., to

increase efficiency and minimize the intracellular accumulation of non-functional mRNA transcripts.

As in the antisense approach, the ribozymes of the invention can be composed of modified oligonucleotides (e.g. for improved stability, targeting, etc.) and should  
5 be delivered to cells which express the polynucleotides of the invention in vivo. DNA constructs encoding the ribozyme may be introduced into the cell in the same manner as described above for the introduction of antisense encoding DNA. A preferred method of delivery involves using a DNA construct "encoding" the ribozyme under the control of a strong constitutive promoter, such as, for example,  
10 pol III or pol II promoter, so that transfected cells will produce sufficient quantities of the ribozyme to destroy endogenous messages and inhibit translation. Since ribozymes unlike antisense molecules, are catalytic, a lower intracellular concentration is required for efficiency.

Antagonist/agonist compounds may be employed to inhibit the cell growth  
15 and proliferation effects of the polypeptides of the present invention on neoplastic cells and tissues, i.e. stimulation of angiogenesis of tumors, and, therefore, retard or prevent abnormal cellular growth and proliferation, for example, in tumor formation or growth.

The antagonist/agonist may also be employed to prevent hyper-vascular  
20 diseases, and prevent the proliferation of epithelial lens cells after extracapsular cataract surgery. Prevention of the mitogenic activity of the polypeptides of the present invention may also be desirous in cases such as restenosis after balloon angioplasty.

The antagonist/agonist may also be employed to prevent the growth of scar  
25 tissue during wound healing.

The antagonist/agonist may also be employed to treat, prevent, and/or diagnose the diseases described herein.

Thus, the invention provides a method of treating or preventing diseases, disorders, and/or conditions, including but not limited to the diseases, disorders,  
5 and/or conditions listed throughout this application, associated with overexpression of a polynucleotide of the present invention by administering to a patient (a) an antisense molecule directed to the polynucleotide of the present invention, and/or (b) a ribozyme directed to the polynucleotide of the present invention, and/or (b) a ribozyme directed to the polynucleotide of the present  
10 invention

#### **Other Activities**

The polypeptide of the present invention, as a result of the ability to stimulate vascular endothelial cell growth, may be employed in treatment for stimulating re-vascularization of ischemic tissues due to various disease conditions such as  
15 thrombosis, arteriosclerosis, and other cardiovascular conditions. These polypeptide may also be employed to stimulate angiogenesis and limb regeneration, as discussed above.

The polypeptide may also be employed for treating wounds due to injuries, burns, post-operative tissue repair, and ulcers since they are mitogenic to various cells  
20 of different origins, such as fibroblast cells and skeletal muscle cells, and therefore, facilitate the repair or replacement of damaged or diseased tissue.

The polypeptide of the present invention may also be employed stimulate neuronal growth and to treat, prevent, and/or diagnose neuronal damage which occurs in certain neuronal disorders or neuro-degenerative conditions such as Alzheimer's  
25 disease, Parkinson's disease, and AIDS-related complex. The polypeptide of the invention may have the ability to stimulate chondrocyte growth, therefore, they may

be employed to enhance bone and periodontal regeneration and aid in tissue transplants or bone grafts.

The polypeptide of the present invention may be also be employed to prevent skin aging due to sunburn by stimulating keratinocyte growth.

5           The polypeptide of the invention may also be employed for preventing hair loss, since FGF family members activate hair-forming cells and promotes melanocyte growth. Along the same lines, the polypeptides of the present invention may be employed to stimulate growth and differentiation of hematopoietic cells and bone marrow cells when used in combination with other cytokines.

10           The polypeptide of the invention may also be employed to maintain organs before transplantation or for supporting cell culture of primary tissues.

The polypeptide of the present invention may also be employed for inducing tissue of mesodermal origin to differentiate in early embryos.

15           The polypeptide or polynucleotides and/or agonist or antagonists of the present invention may also increase or decrease the differentiation or proliferation of embryonic stem cells, besides, as discussed above, hematopoietic lineage.

          The polypeptide or polynucleotides and/or agonist or antagonists of the present invention may also be used to modulate mammalian characteristics, such as  
20   body height, weight, hair color, eye color, skin, percentage of adipose tissue, pigmentation, size, and shape (e.g., cosmetic surgery). Similarly, polypeptides or polynucleotides and/or agonist or antagonists of the present invention may be used to modulate mammalian metabolism affecting catabolism, anabolism, processing, utilization, and storage of energy.

25           Polypeptide or polynucleotides and/or agonist or antagonists of the present invention may be used to change a mammal's mental state or physical state by

influencing biorhythms, cardiac rhythms, depression (including depressive diseases, disorders, and/or conditions), tendency for violence, tolerance for pain, reproductive capabilities (preferably by Activin or Inhibin-like activity), hormonal or endocrine levels, appetite, libido, memory, stress, or other cognitive qualities.

- 5            Polypeptide or polynucleotides and/or agonist or antagonists of the present invention may also be used as a food additive or preservative, such as to increase or decrease storage capabilities, fat content, lipid, protein, carbohydrate, vitamins, minerals, cofactors or other nutritional components.

#### 10    **Other Preferred Embodiments**

Other preferred embodiments of the claimed invention include an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least about 50 contiguous nucleotides in the nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table 1.

- 15           Also preferred is a nucleic acid molecule wherein said sequence of contiguous nucleotides is included in the nucleotide sequence of SEQ ID NO:X in the range of positions beginning with the nucleotide at about the position of the 5' Nucleotide of the Clone Sequence and ending with the nucleotide at about the position of the 3' Nucleotide of the Clone Sequence as defined for SEQ ID NO:X in Table 1.

- 20           Also preferred is a nucleic acid molecule wherein said sequence of contiguous nucleotides is included in the nucleotide sequence of SEQ ID NO:X in the range of positions beginning with the nucleotide at about the position of the 5' Nucleotide of the Start Codon and ending with the nucleotide at about the position of the 3' Nucleotide of the Clone Sequence as defined for SEQ ID NO:X in Table 1.

- 25           Similarly preferred is a nucleic acid molecule wherein said sequence of contiguous nucleotides is included in the nucleotide sequence of SEQ ID NO:X in the

range of positions beginning with the nucleotide at about the position of the 5' Nucleotide of the First Amino Acid of the Signal Peptide and ending with the nucleotide at about the position of the 3' Nucleotide of the Clone Sequence as defined for SEQ ID NO:X in Table 1.

- 5           Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least about 150 contiguous nucleotides in the nucleotide sequence of SEQ ID NO:X.

- Further preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least about 500  
10 contiguous nucleotides in the nucleotide sequence of SEQ ID NO:X.

- A further preferred embodiment is a nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to the nucleotide sequence of SEQ ID NO:X beginning with the nucleotide at about the position of the 5' Nucleotide of the First Amino Acid of the Signal Peptide and ending with the nucleotide at about  
15 the position of the 3' Nucleotide of the Clone Sequence as defined for SEQ ID NO:X in Table 1.

          A further preferred embodiment is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to the complete nucleotide sequence of SEQ ID NO:X.

- 20           Also preferred is an isolated nucleic acid molecule which hybridizes under stringent hybridization conditions to a nucleic acid molecule, wherein said nucleic acid molecule which hybridizes does not hybridize under stringent hybridization conditions to a nucleic acid molecule having a nucleotide sequence consisting of only A residues or of only T residues.

- 25           Also preferred is a composition of matter comprising a DNA molecule which comprises a human cDNA clone identified by a cDNA Clone Identifier in Table 1,



which DNA molecule is contained in the material deposited with the American Type Culture Collection and given the ATCC Deposit Number shown in Table 1 for said cDNA Clone Identifier.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide  
5 sequence which is at least 95% identical to a sequence of at least 50 contiguous nucleotides in the nucleotide sequence of a human cDNA clone identified by a cDNA Clone Identifier in Table 1, which DNA molecule is contained in the deposit given the ATCC Deposit Number shown in Table 1.

Also preferred is an isolated nucleic acid molecule, wherein said sequence of  
10 at least 50 contiguous nucleotides is included in the nucleotide sequence of the complete open reading frame sequence encoded by said human cDNA clone.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to sequence of at least 150 contiguous nucleotides in the nucleotide sequence encoded by said human cDNA clone.

15 A further preferred embodiment is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to sequence of at least 500 contiguous nucleotides in the nucleotide sequence encoded by said human cDNA clone.

A further preferred embodiment is an isolated nucleic acid molecule  
20 comprising a nucleotide sequence which is at least 95% identical to the complete nucleotide sequence encoded by said human cDNA clone.

A further preferred embodiment is a method for detecting in a biological sample a nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence  
25 selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table 1; and a nucleotide sequence encoded by

a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1; which method comprises a step of comparing a nucleotide sequence of at least one nucleic acid molecule in said sample with a sequence selected from said group and  
5 determining whether the sequence of said nucleic acid molecule in said sample is at least 95% identical to said selected sequence.

Also preferred is the above method wherein said step of comparing sequences comprises determining the extent of nucleic acid hybridization between nucleic acid molecules in said sample and a nucleic acid molecule comprising said sequence  
10 selected from said group. Similarly, also preferred is the above method wherein said step of comparing sequences is performed by comparing the nucleotide sequence determined from a nucleic acid molecule in said sample with said sequence selected from said group. The nucleic acid molecules can comprise DNA molecules or RNA molecules.

15 A further preferred embodiment is a method for identifying the species, tissue or cell type of a biological sample which method comprises a step of detecting nucleic acid molecules in said sample, if any, comprising a nucleotide sequence that is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X  
20 wherein X is any integer as defined in Table 1; and a nucleotide sequence encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

The method for identifying the species, tissue or cell type of a biological  
25 sample can comprise a step of detecting nucleic acid molecules comprising a nucleotide sequence in a panel of at least two nucleotide sequences, wherein at least

one sequence in said panel is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from said group.

Also preferred is a method for diagnosing in a subject a pathological condition associated with abnormal structure or expression of a gene encoding a secreted protein identified in Table 1, which method comprises a step of detecting in a biological sample obtained from said subject nucleic acid molecules, if any, comprising a nucleotide sequence that is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table 1; and a nucleotide sequence encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

The method for diagnosing a pathological condition can comprise a step of detecting nucleic acid molecules comprising a nucleotide sequence in a panel of at least two nucleotide sequences, wherein at least one sequence in said panel is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from said group.

Also preferred is a composition of matter comprising isolated nucleic acid molecules wherein the nucleotide sequences of said nucleic acid molecules comprise a panel of at least two nucleotide sequences, wherein at least one sequence in said panel is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table 1; and a nucleotide sequence encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA

clone in Table 1. The nucleic acid molecules can comprise DNA molecules or RNA molecules.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 90% identical to a sequence of at least about 10 contiguous amino acids in the amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1.

Also preferred is a polypeptide, wherein said sequence of contiguous amino acids is included in the amino acid sequence of SEQ ID NO:Y in the range of positions beginning with the residue at about the position of the First Amino Acid of the Secreted Portion and ending with the residue at about the Last Amino Acid of the Open Reading Frame as set forth for SEQ ID NO:Y in Table 1.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 30 contiguous amino acids in the amino acid sequence of SEQ ID NO:Y.

Further preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 100 contiguous amino acids in the amino acid sequence of SEQ ID NO:Y.

Further preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to the complete amino acid sequence of SEQ ID NO:Y.

Further preferred is an isolated polypeptide comprising an amino acid sequence at least 90% identical to a sequence of at least about 10 contiguous amino acids in the complete amino acid sequence of a secreted protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is a polypeptide wherein said sequence of contiguous amino acids is included in the amino acid sequence of a secreted portion of the secreted

protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is an isolated polypeptide comprising an amino acid sequence  
5 at least 95% identical to a sequence of at least about 30 contiguous amino acids in the amino acid sequence of the secreted portion of the protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is an isolated polypeptide comprising an amino acid sequence  
10 at least 95% identical to a sequence of at least about 100 contiguous amino acids in the amino acid sequence of the secreted portion of the protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is an isolated polypeptide comprising an amino acid sequence  
15 at least 95% identical to the amino acid sequence of the secreted portion of the protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Further preferred is an isolated antibody which binds specifically to a  
20 polypeptide comprising an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained  
25 in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Further preferred is a method for detecting in a biological sample a polypeptide comprising an amino acid sequence which is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as  
5 defined in Table 1; and a complete amino acid sequence of a protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1; which method comprises a step of comparing an amino acid sequence of at least one polypeptide molecule in said sample with a sequence selected from said group  
10 and determining whether the sequence of said polypeptide molecule in said sample is at least 90% identical to said sequence of at least 10 contiguous amino acids.

Also preferred is the above method wherein said step of comparing an amino acid sequence of at least one polypeptide molecule in said sample with a sequence selected from said group comprises determining the extent of specific binding of  
15 polypeptides in said sample to an antibody which binds specifically to a polypeptide comprising an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a protein encoded by a human cDNA  
20 clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is the above method wherein said step of comparing sequences is performed by comparing the amino acid sequence determined from a polypeptide molecule in said sample with said sequence selected from said group.

25 Also preferred is a method for identifying the species, tissue or cell type of a biological sample which method comprises a step of detecting polypeptide molecules

in said sample, if any, comprising an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a secreted protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is the above method for identifying the species, tissue or cell type of a biological sample, which method comprises a step of detecting polypeptide molecules comprising an amino acid sequence in a panel of at least two amino acid sequences, wherein at least one sequence in said panel is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the above group.

Also preferred is a method for diagnosing in a subject a pathological condition associated with abnormal structure or expression of a gene encoding a secreted protein identified in Table 1, which method comprises a step of detecting in a biological sample obtained from said subject polypeptide molecules comprising an amino acid sequence in a panel of at least two amino acid sequences, wherein at least one sequence in said panel is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a secreted protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

In any of these methods, the step of detecting said polypeptide molecules includes using an antibody.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a nucleotide sequence encoding a polypeptide wherein said polypeptide comprises an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence  
5 selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a secreted protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

10 Also preferred is an isolated nucleic acid molecule, wherein said nucleotide sequence encoding a polypeptide has been optimized for expression of said polypeptide in a prokaryotic host.

Also preferred is an isolated nucleic acid molecule, wherein said polypeptide comprises an amino acid sequence selected from the group consisting of: an amino  
15 acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a secreted protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Further preferred is a method of making a recombinant vector comprising  
20 inserting any of the above isolated nucleic acid molecule into a vector. Also preferred is the recombinant vector produced by this method. Also preferred is a method of making a recombinant host cell comprising introducing the vector into a host cell, as well as the recombinant host cell produced by this method.

Also preferred is a method of making an isolated polypeptide comprising  
25 culturing this recombinant host cell under conditions such that said polypeptide is expressed and recovering said polypeptide. Also preferred is this method of making



an isolated polypeptide, wherein said recombinant host cell is a eukaryotic cell and said polypeptide is a secreted portion of a human secreted protein comprising an amino acid sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y beginning with the residue at the position of the First Amino Acid of the Secreted Portion of SEQ ID NO:Y wherein Y is an integer set forth in Table 1 and said position of the First Amino Acid of the Secreted Portion of SEQ ID NO:Y is defined in Table 1; and an amino acid sequence of a secreted portion of a protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1. The isolated polypeptide produced by this method is also preferred.

Also preferred is a method of treatment of an individual in need of an increased level of a secreted protein activity, which method comprises administering to such an individual a pharmaceutical composition comprising an amount of an isolated polypeptide, polynucleotide, or antibody of the claimed invention effective to increase the level of said protein activity in said individual.

The above-recited applications have uses in a wide variety of hosts. Such hosts include, but are not limited to, human, murine, rabbit, goat, guinea pig, camel, horse, mouse, rat, hamster, pig, micro-pig, chicken, goat, cow, sheep, dog, cat, non-human primate, and human. In specific embodiments, the host is a mouse, rabbit, goat, guinea pig, chicken, rat, hamster, pig, sheep, dog or cat. In preferred embodiments, the host is a mammal. In most preferred embodiments, the host is a human.

In specific embodiments of the invention, for each "Contig ID" listed in the fourth column of Table 2, preferably excluded are one or more polynucleotides comprising, or alternatively consisting of, a nucleotide sequence referenced in the fifth column of Table 2 and described by the general formula of a-b, whereas a and b

are uniquely determined for the corresponding SEQ ID NO:X referred to in column 3 of Table 2. Further specific embodiments are directed to polynucleotide sequences excluding one, two, three, four, or more of the specific polynucleotide sequences referred to in the fifth column of Table 2. In no way is this listing meant to encompass all of the sequences which may be excluded by the general formula, it is just a representative example. All references available through these accessions are hereby incorporated by reference in their entirety.

TABLE 2

Gene No.	cDNA Clone ID	NT SEQ ID NO: X	Contig ID	Public Accession Numbers
9	HNSAD53	19	850871	AA099387, AA099388, AA255541, AA256657
12	HSSGD52	22	845666	T75428, R19112, R44778, R48178, R48179, R44778, R69447, R70430, H42841, R82965, H99336, N26442, N93753, W44908, W45166, W52643, AA127685, AA127785, AA541734, AA559052, AA577454, AA837786, AA857204, AA938585, AA436865, D11951, AA442622, AA447921, AA678751, AA843629, AA861929, AA905576, AA917796, AA972078, A1084548, T10746
13	HAPSA79	23	846517	T78682, R48289, R48391, R49179, R49179, H42085, R90877, H71948, H73479, H75569, W24023, W60631, W60630, W74364, W76344, AA028967, AA029134, AA043327, AA043328, AA082108, AA131883, AA131882, AA146937, AA149513, AA149644, AA502173, AA588293, AA810705, AA931102, AA995574, W29012, AA404374, AA635131, AA733045, AA780109, AA905202, AA928795, AA978196, A1089282, A1092790, Z26990, T24058, Z38370, Z42102
13	HAPSA79	73	887467	T78682, R48289, R48391, R49179, R49179, H42085, R90877, H71948, H73479, H75569, W24023, W60631, W60630, W74364, W76344, AA028967, AA029134, AA043327, AA043328, AA082108, AA131883, AA131882, AA146937, AA149513, AA149644, AA502173, AA588293, AA810705, AA931102, AA995574, W29012, AA404374, AA635131, AA733045, AA780109, AA905202, AA928795, AA978196, A1089282, A1092790, Z26990, T24058, Z38370, Z42102, A1439728, A1440039, A1571763, A1131104, A1131505, A1139050, A1144018, A1146486,

				AI147653, AI282048, AI312742, AI342567, AI350488, AI351676
13	HAPSA79	74	878627	T78682, R48289, R48391, R49179, R49179, H42085, R90877, H71948, H73479, H75569, W24023, W60631, W60630, W74364, W76344, AA028967, AA029134, AA043327, AA043328, AA082108, AA131883, AA131882, AA146937, AA149513, AA149644, AA502173, AA588293, AA810705, AA931102, AA995574, W29012, AA404374, AA635131, AA733045, AA780109, AA905202, AA928795, AA978196, AI089282, AI092790, Z26990, T24058, Z38370, Z42102, AI439728, AI440039, AI571763, AI131104, AI131505, AI139050, AI144018, AI146486, AI147653, AI282048, AI312742, AI342567, AI350488, AI351676
14	HASAU84	24	846116	T54072, R99331, R99423, H92966, H94196, H98910, N24243, N71838, N76693, W25638, AA280637, AA282003, AA287775, AA761419, AA975590, C03884, AI095435, AI095873
18	HTLFE57	28	791409	T61071, T61174, T65029, T85783, R51375, R64311, H19641, H20476, H20475, H41099, N23943, N31844, N72499, W31498, U55991, W58172, W58112, W72743, AA018419, AA041503, AA056110, AA058572, AA132154, AA210767, AA259063
19	HTADW91	29	844835	R73343, AA427824, AA429042, AA627105, AA894462, AA434527
23	HEGAK44	33	852219	H22619, H28173, H30770, H38160, H38161, H47626, H47928, R83451, R90899, R90900, H77842, H98457, N20006, N62472, N68589, N70064, N79192, N91977, W24308, W51878, W69157, W69283, W86278, W86322, AA010010, AA010947, AA011331, AA025439, AA025756, AA040784, AA041220, AA045200, AA045402, AA127325, AA132774, AA151322, AA151323, AA235078, AA235187, AA243612, AA256598, AA283142, AA493689, AA515704, AA523381, AA534998, AA584247, AA632248, AA740359, AA742625, AA761102, AA769133, AA805765, AA805772, AA828160, AA916415, AA936239, AA939010, AA975682, AA975683, AI094486, W26195, AA641790, AA641820, AA443285, AA476701, AA598872, AA628489, AA628769, AA633800, AA703908, AA709276, AA774823, AA777561, AA782472, AA846241, AA984395, AA992550, AI016471, AI016482, AI025758, AI075853, AI041370, Z38476, AA694293
23	HEGAK44	76	859272	H22619, H28173, H30770, H38160, H38161, H47626, H47928, R83451, R90899, R90900, H77842, H98457, N20006, N62472, N68589, N70064, N79192, N91977, W24308, W51878, W69157, W69283, W86278, W86322, AA010010, AA010947, AA011331, AA025439, AA025756, AA040784, AA041220, AA045200, AA045402, AA127325, AA132774, AA151322, AA151323, AA235078, AA235187, AA243612, AA256598,

				AA283142, AA493689, AA515704, AA523381, AA534998, AA584247, AA632248, AA740359, AA742625, AA761102, AA769133, AA805765, AA805772, AA828160, AA916415, AA936239, AA939010, AA975682, AA975683, AI094486, W26195, AA641790, AA641820, AA443285, AA476701, AA598872, AA628489, AA628769, AA633800, AA703908, AA709276, AA774823, AA777561, AA782472, AA846241, AA984395, AA992550, AI016471, AI016482, AI025758, AI075853, AI041370, Z38476, AA694293
25	HBAFV19	35	843036	AA480361, AA886008, AA704856, AI050770
26	HTXDO17	36	845132	W92862, W92861, AA064689, AA064647
26	HTXDO17	77	843388	W92862, W92861, AA064689, AA064647
36	HBIBB20	78	897484	T55730, T55772, R10660, R38308, R40971, R51590, R53750, R53751, R40971, H42344, H43116, H45702, H45753, H52665, AA025730, AA037073, AA134803, AA134802, AA143267, AA233548, AA233662, AA460484, AA429477, AA230095, AA514696, AA922522, AA932271, AI074169, AA284706, AA287135, AA291748, AA291701, AA443486, AA455817, AA626289, AA722825, T03319, AA984833, AI003456, AI074545, AI042088, Z39044, Z42923, F04029, AI439151, AI560594, AI141636, AI659329, AI537969, AI278324, AI669079
39	HTLIQ05	49	845671	AA290985, AA290868, AA291047, AA481857, AI073890
40	HTGAM78	50	842530	AA774076
42	HWBFX31	52	799427	H93613, N75773
44	HSIDL71	79	753398	T61540, R11626, H30182, R83928, AA193088
45	HOVBX78	55	845686	R13128, R14622, R17840, R20573, N43756, D82396, D82213, F06294, F07508, F08130
47	HWADJ89	57	799506	T78828, R34372, R34492, R34886, R49268, R49268, R72500, N35410, N48042, N55117, N62863, N73777, N77769, N99398, AA040909, AA135712
48	HYABE50	81	843598	T98923, R32291, R32334, R61350, H00442, H00483, H06504, H06560, R98051, H70361, H75780, H95563, N23992, N41042, N49042, N49106, N74127, N75739, W01952, W56287, N90675, AA149983, AA152140, AA193577, AA236205, AA253129, AA257087, AA257117, AA255440, AA428590, AA429544, AA524252, AA559118, AA603712, AA746125, AA765360, AA808752, W26733, C17503, AA400203, AA400365, AA410306, AA457695, AA480802, AA481337, AA775698, AA775773, AA781085, AA788653, AA772768, AI015261, AI032934, AI093127, T10771, Z40471, Z40711, Z44161, F07055, AA694547
55	HFCFJ18	82	648525	T71064, R01186, R01297, H26338, R92098, N27661, N39169, N45245, N54172, W81557, W81556, W90445, W90232, AA007609, AA010652, AA026127, AA026594, AA055332, AA055929, AA085984, AA086026, AA428936

				AA429151
56	HANGG89	66	845690	R12712, R39765, R39766, R42588, R42588, H18309, H47347, H47436, H64403, H64415, H64452, H64466, H65317, H90657, H91585, H64403, N30904, W88754, W88860, AA011390, AA011427, AA962561, AA479629, AA477305, AI040619
56	HEOMP42	83	852533	T50378, T51069, T87331, T87432, T80282, R16493, R16793, R16889, R16943, R20740, R22221, R22276, R27597, R27696, R36581, R36582, R39968, R43467, R49072, R49692, R52816, R52851, R49072, R43467, R43494, R20740, R46644, R39968, R49692, R62955, R63008, R66425, R66819, R67917, H21718, H27063, H30186, H42692, H39131, H43926, H44586, H46389, R99312, R99404, H64752, H64751, H84964, H97190, H64752, H97608, N26236, N28697, N33047, N33556, N41538, N42109, N70229, N79806, N98255, W01018, W19986, W44335, W44449, W81010, W81009, W90684, W90779, W99356, W99386, N90413, AA009837, AA010386, AA010322, AA019377, AA019378, AA026243, AA029623, AA033587, AA033588, AA056089, AA079689, AA081447, AA081752, AA082528, AA084728, AA100074, AA100159, AA113183, AA127588, AA156575, AA161199, AA161200, AA181973, AA226873, AA226833, AA233473, AA234238, AA235690, AA256297, AA256403, AA417901, AA430035, AA430244, AA492600, AA279831, AA468684, AA506011, AA506624, AA514562, AA587048, AA595566, AA604178, AA631096, AA573619, AA575906, AA579642, AA565772, AA714575, AA730078, AA742696, AA765252, AA812238, AA826845, AA904238, AA918044, AA922424, AA923043, AA948416, F19046, D81115, D81226, D81251, AI094829, N55823, N56294, W38309, N83234, C15348, C15347, AA094528, AA214295, AA410543, AA449260, AA449391, AA453781, AA453865, AA486791, AA486966, AA629206, AA633802, AA663961, AA664054, AA664222, AA447616, AA448013, AA405294, AA405295, AA707053, AA708207, AA708246, AA719821, AA770136, AA776635, AA777143, AA843836, AA855102, AA860518, AA961051, AA984117, AA984677, AA992009, AA938909, AI022865, AI031958, AI037972, AI052488, AI074677, AI076586, AI077444, AI077446, AI040543, AI080347, AI123047, T15853, T23520, Z39649, F00742, AA772860, AA693407
56	HPRAL78	84	844216	T52017, T63960, R36715, R51437, H12158, H66782, H66783, AA043375, AA081115, AA081290, AA081697, AA082047, AA251006, AA424398, AA506965, AA508725, AA554343, AA588312, AA604134, AA612772, AA766464, AA832062, AA832305, W22554, C04045, AA757547, AI055912, AI076726, AI095534,

				AA702770
56	HDTAT90	85	692291	R60726, R90863
58	HWLQU40	68	799425	H45996, H50224, W04634

Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not intended as limiting.

5

### Examples

#### Example 1: Isolation of a Selected cDNA Clone From the Deposited Sample

Each cDNA clone in a cited ATCC deposit is contained in a plasmid vector.

- 10 Table 1 identifies the vectors used to construct the cDNA library from which each clone was isolated. In many cases, the vector used to construct the library is a phage vector from which a plasmid has been excised. The table immediately below correlates the related plasmid for each phage vector used in constructing the cDNA library. For example, where a particular clone is identified in Table 1 as being
- 15 isolated in the vector "Lambda Zap," the corresponding deposited clone is in "pBluescript."

	<u>Vector Used to Construct Library</u>	<u>Corresponding Deposited</u>
	<u>Plasmid</u>	
	Lambda Zap	pBluescript (pBS)
20	Uni-Zap XR	pBluescript (pBS)
	Zap Express	pBK
	lafmid BA	plafmid BA
	pSport1	pSport1
	pCMVSport 2.0	pCMVSport 2.0
25	pCMVSport 3.0	pCMVSport 3.0
	pCR®2.1	pCR®2.1

Vectors Lambda Zap (U.S. Patent Nos. 5,128,256 and 5,286,636), Uni-Zap XR (U.S. Patent Nos. 5,128, 256 and 5,286,636), Zap Express (U.S. Patent Nos. 5,128,256 and 5,286,636), pBluescript (pBS) (Short, J. M. et al., Nucleic Acids Res. 16:7583-7600 (1988); Alting-Mees, M. A. and Short, J. M., Nucleic Acids Res. 17:9494 (1989)) and pBK (Alting-Mees, M. A. et al., Strategies 5:58-61 (1992)) are commercially available from Stratagene Cloning Systems, Inc., 11011 N. Torrey Pines Road, La Jolla, CA, 92037. pBS contains an ampicillin resistance gene and pBK contains a neomycin resistance gene. Both can be transformed into E. coli strain XL-1 Blue, also available from Stratagene. pBS comes in 4 forms SK+, SK-, KS+ and KS. The S and K refers to the orientation of the polylinker to the T7 and T3 primer sequences which flank the polylinker region ("S" is for SacI and "K" is for KpnI which are the first sites on each respective end of the linker). "+" or "-" refer to the orientation of the f1 origin of replication ("ori"), such that in one orientation, single stranded rescue initiated from the f1 ori generates sense strand DNA and in the other, antisense.

Vectors pSport1, pCMVSPORT 2.0 and pCMVSPORT 3.0, were obtained from Life Technologies, Inc., P. O. Box 6009, Gaithersburg, MD 20897. All Sport vectors contain an ampicillin resistance gene and may be transformed into E. coli strain DH10B, also available from Life Technologies. (See, for instance, Gruber, C. E., et al., Focus 15:59 (1993).) Vector lacmid BA (Bento Soares, Columbia University, NY) contains an ampicillin resistance gene and can be transformed into E. coli strain XL-1 Blue. Vector pCR<sup>®</sup>2.1, which is available from Invitrogen, 1600 Faraday Avenue, Carlsbad, CA 92008, contains an ampicillin resistance gene and may be transformed into E. coli strain DH10B, available from Life Technologies. (See, for instance, Clark, J. M., Nuc. Acids Res. 16:9677-9686 (1988) and Mead, D. et al., Bio/Technology 9: (1991).) Preferably, a polynucleotide of the present invention



does not comprise the phage vector sequences identified for the particular clone in Table 1, as well as the corresponding plasmid vector sequences designated above.

The deposited material in the sample assigned the ATCC Deposit Number cited in Table 1 for any given cDNA clone also may contain one or more additional  
5 plasmids, each comprising a cDNA clone different from that given clone. Thus, deposits sharing the same ATCC Deposit Number contain at least a plasmid for each cDNA clone identified in Table 1. Typically, each ATCC deposit sample cited in Table 1 comprises a mixture of approximately equal amounts (by weight) of about 50 plasmid DNAs, each containing a different cDNA clone; but such a deposit sample  
10 may include plasmids for more or less than 50 cDNA clones, up to about 500 cDNA clones.

Two approaches can be used to isolate a particular clone from the deposited sample of plasmid DNAs cited for that clone in Table 1. First, a plasmid is directly isolated by screening the clones using a polynucleotide probe corresponding to SEQ  
15 ID NO:X.

Particularly, a specific polynucleotide with 30-40 nucleotides is synthesized using an Applied Biosystems DNA synthesizer according to the sequence reported. The oligonucleotide is labeled, for instance, with  $^{32}\text{P}$ - $\gamma$ -ATP using T4 polynucleotide kinase and purified according to routine methods. (E.g., Maniatis et al., Molecular  
20 Cloning: A Laboratory Manual, Cold Spring Harbor Press, Cold Spring, NY (1982).) The plasmid mixture is transformed into a suitable host, as indicated above (such as XL-1 Blue (Stratagene)) using techniques known to those of skill in the art, such as those provided by the vector supplier or in related publications or patents cited above. The transformants are plated on 1.5% agar plates (containing the appropriate selection  
25 agent, e.g., ampicillin) to a density of about 150 transformants (colonies) per plate. These plates are screened using Nylon membranes according to routine methods for

bacterial colony screening (e.g., Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2nd Edit., (1989), Cold Spring Harbor Laboratory Press, pages 1.93 to 1.104), or other techniques known to those of skill in the art.

Alternatively, two primers of 17-20 nucleotides derived from both ends of the  
5 SEQ ID NO:X (i.e., within the region of SEQ ID NO:X bounded by the 5' NT and  
the 3' NT of the clone defined in Table 1) are synthesized and used to amplify the  
desired cDNA using the deposited cDNA plasmid as a template. The polymerase  
chain reaction is carried out under routine conditions, for instance, in 25 ul of reaction  
mixture with 0.5 ug of the above cDNA template. A convenient reaction mixture is  
10 1.5-5 mM MgCl<sub>2</sub>, 0.01% (w/v) gelatin, 20 uM each of dATP, dCTP, dGTP, dTTP, 25  
pmol of each primer and 0.25 Unit of Taq polymerase. Thirty five cycles of PCR  
(denaturation at 94 degree C for 1 min; annealing at 55 degree C for 1 min; elongation  
at 72 degree C for 1 min) are performed with a Perkin-Elmer Cetus automated  
thermal cycler. The amplified product is analyzed by agarose gel electrophoresis and  
15 the DNA band with expected molecular weight is excised and purified. The PCR  
product is verified to be the selected sequence by subcloning and sequencing the  
DNA product.

Several methods are available for the identification of the 5' or 3' non-coding  
portions of a gene which may not be present in the deposited clone. These methods  
20 include but are not limited to, filter probing, clone enrichment using specific probes,  
and protocols similar or identical to 5' and 3' "RACE" protocols which are well  
known in the art. For instance, a method similar to 5' RACE is available for  
generating the missing 5' end of a desired full-length transcript. (Fromont-Racine et  
al., *Nucleic Acids Res.* 21(7):1683-1684 (1993).)

25 Briefly, a specific RNA oligonucleotide is ligated to the 5' ends of a  
population of RNA presumably containing full-length gene RNA transcripts. A

primer set containing a primer specific to the ligated RNA oligonucleotide and a primer specific to a known sequence of the gene of interest is used to PCR amplify the 5' portion of the desired full-length gene. This amplified product may then be sequenced and used to generate the full length gene.

- 5           This above method starts with total RNA isolated from the desired source, although poly-A+ RNA can be used. The RNA preparation can then be treated with phosphatase if necessary to eliminate 5' phosphate groups on degraded or damaged RNA which may interfere with the later RNA ligase step. The phosphatase should then be inactivated and the RNA treated with tobacco acid pyrophosphatase in order
- 10   to remove the cap structure present at the 5' ends of messenger RNAs. This reaction leaves a 5' phosphate group at the 5' end of the cap cleaved RNA which can then be ligated to an RNA oligonucleotide using T4 RNA ligase.

- This modified RNA preparation is used as a template for first strand cDNA synthesis using a gene specific oligonucleotide. The first strand synthesis reaction is
- 15   used as a template for PCR amplification of the desired 5' end using a primer specific to the ligated RNA oligonucleotide and a primer specific to the known sequence of the gene of interest. The resultant product is then sequenced and analyzed to confirm that the 5' end sequence belongs to the desired gene.

20   **Example 2: Isolation of Genomic Clones Corresponding to a Polynucleotide**

          A human genomic P1 library (Genomic Systems, Inc.) is screened by PCR using primers selected for the cDNA sequence corresponding to SEQ ID NO:X., according to the method described in Example 1. (See also, Sambrook.)

25   **Example 3: Tissue Distribution of Polypeptide**

Tissue distribution of mRNA expression of polynucleotides of the present invention is determined using protocols for Northern blot analysis, described by, among others, Sambrook et al. For example, a cDNA probe produced by the method described in Example 1 is labeled with  $P^{32}$  using the rediprime™ DNA labeling system (Amersham Life Science), according to manufacturer's instructions. After labeling, the probe is purified using CHROMA SPIN-100™ column (Clontech Laboratories, Inc.), according to manufacturer's protocol number PT1200-1. The purified labeled probe is then used to examine various human tissues for mRNA expression.

10 Multiple Tissue Northern (MTN) blots containing various human tissues (H) or human immune system tissues (IM) (Clontech) are examined with the labeled probe using ExpressHyb™ hybridization solution (Clontech) according to manufacturer's protocol number PT1190-1. Following hybridization and washing, the blots are mounted and exposed to film at -70 degree C overnight, and the films  
15 developed according to standard procedures.

#### **Example 4: Chromosomal Mapping of the Polynucleotides**

An oligonucleotide primer set is designed according to the sequence at the 5' end of SEQ ID NO:X. This primer preferably spans about 100 nucleotides. This  
20 primer set is then used in a polymerase chain reaction under the following set of conditions : 30 seconds, 95 degree C; 1 minute, 56 degree C; 1 minute, 70 degree C. This cycle is repeated 32 times followed by one 5 minute cycle at 70 degree C. Human, mouse, and hamster DNA is used as template in addition to a somatic cell hybrid panel containing individual chromosomes or chromosome fragments (Bios,  
25 Inc). The reactions is analyzed on either 8% polyacrylamide gels or 3.5 % agarose

gels. Chromosome mapping is determined by the presence of an approximately 100 bp PCR fragment in the particular somatic cell hybrid.

#### **Example 5: Bacterial Expression of a Polypeptide**

5           A polynucleotide encoding a polypeptide of the present invention is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' ends of the DNA sequence, as outlined in Example 1, to synthesize insertion fragments. The primers used to amplify the cDNA insert should preferably contain restriction sites, such as BamHI and XbaI, at the 5' end of the primers in order to clone the amplified product  
10   into the expression vector. For example, BamHI and XbaI correspond to the restriction enzyme sites on the bacterial expression vector pQE-9. (Qiagen, Inc., Chatsworth, CA). This plasmid vector encodes antibiotic resistance (Amp<sup>r</sup>), a bacterial origin of replication (ori), an IPTG-regulatable promoter/operator (P/O), a ribosome binding site (RBS), a 6-histidine tag (6-His), and restriction enzyme cloning  
15   sites.

          The pQE-9 vector is digested with BamHI and XbaI and the amplified fragment is ligated into the pQE-9 vector maintaining the reading frame initiated at the bacterial RBS. The ligation mixture is then used to transform the E. coli strain M15/rep4 (Qiagen, Inc.) which contains multiple copies of the plasmid pREP4, which  
20   expresses the lacI repressor and also confers kanamycin resistance (Kan<sup>r</sup>). Transformants are identified by their ability to grow on LB plates and ampicillin/kanamycin resistant colonies are selected. Plasmid DNA is isolated and confirmed by restriction analysis.

          Clones containing the desired constructs are grown overnight (O/N) in liquid  
25   culture in LB media supplemented with both Amp (100 ug/ml) and Kan (25 ug/ml). The O/N culture is used to inoculate a large culture at a ratio of 1:100 to 1:250. The

cells are grown to an optical density 600 (O.D.<sup>600</sup>) of between 0.4 and 0.6. IPTG (Isopropyl-B-D-thiogalacto pyranoside) is then added to a final concentration of 1 mM. IPTG induces by inactivating the lacI repressor, clearing the P/O leading to increased gene expression.

5           Cells are grown for an extra 3 to 4 hours. Cells are then harvested by centrifugation (20 mins at 6000Xg). The cell pellet is solubilized in the chaotropic agent 6 Molar Guanidine HCl by stirring for 3-4 hours at 4 degree C. The cell debris is removed by centrifugation, and the supernatant containing the polypeptide is loaded onto a nickel-nitrilo-tri-acetic acid ("Ni-NTA") affinity resin column (available from  
10   QIAGEN, Inc., *supra*). Proteins with a 6 x His tag bind to the Ni-NTA resin with high affinity and can be purified in a simple one-step procedure (for details see: The QIAexpressionist (1995) QIAGEN, Inc., *supra*).

Briefly, the supernatant is loaded onto the column in 6 M guanidine-HCl, pH 8, the column is first washed with 10 volumes of 6 M guanidine-HCl, pH 8, then  
15   washed with 10 volumes of 6 M guanidine-HCl pH 6, and finally the polypeptide is eluted with 6 M guanidine-HCl, pH 5.

The purified protein is then renatured by dialyzing it against phosphate-buffered saline (PBS) or 50 mM Na-acetate, pH 6 buffer plus 200 mM NaCl. Alternatively, the protein can be successfully refolded while immobilized on the Ni-  
20   NTA column. The recommended conditions are as follows: renature using a linear 6M-1M urea gradient in 500 mM NaCl, 20% glycerol, 20 mM Tris/HCl pH 7.4, containing protease inhibitors. The renaturation should be performed over a period of 1.5 hours or more. After renaturation the proteins are eluted by the addition of 250 mM imidazole. Imidazole is removed by a final dialyzing step against PBS or 50  
25   mM sodium acetate pH 6 buffer plus 200 mM NaCl. The purified protein is stored at 4 degree C or frozen at -80 degree C.

In addition to the above expression vector, the present invention further includes an expression vector comprising phage operator and promoter elements operatively linked to a polynucleotide of the present invention, called pHE4a. (ATCC Accession Number 209645, deposited on February 25, 1998.) This vector contains:

5 1) a neomycinphosphotransferase gene as a selection marker, 2) an *E. coli* origin of replication, 3) a T5 phage promoter sequence, 4) two lac operator sequences, 5) a Shine-Delgarno sequence, and 6) the lactose operon repressor gene (*lacIq*). The origin of replication (*oriC*) is derived from pUC19 (LTI, Gaithersburg, MD). The promoter sequence and operator sequences are made synthetically.

10 DNA can be inserted into the pHEa by restricting the vector with *NdeI* and *XbaI*, *BamHI*, *XhoI*, or *Asp718*, running the restricted product on a gel, and isolating the larger fragment (the stuffer fragment should be about 310 base pairs). The DNA insert is generated according to the PCR protocol described in Example 1, using PCR primers having restriction sites for *NdeI* (5' primer) and *XbaI*, *BamHI*, *XhoI*, or

15 *Asp718* (3' primer). The PCR insert is gel purified and restricted with compatible enzymes. The insert and vector are ligated according to standard protocols.

The engineered vector could easily be substituted in the above protocol to express protein in a bacterial system.

#### 20 **Example 6: Purification of a Polypeptide from an Inclusion Body**

The following alternative method can be used to purify a polypeptide expressed in *E. coli* when it is present in the form of inclusion bodies. Unless otherwise specified, all of the following steps are conducted at 4-10 degree C.

Upon completion of the production phase of the *E. coli* fermentation, the cell

25 culture is cooled to 4-10 degree C and the cells harvested by continuous centrifugation at 15,000 rpm (Heraeus Sepatech). On the basis of the expected yield

of protein per unit weight of cell paste and the amount of purified protein required, an appropriate amount of cell paste, by weight, is suspended in a buffer solution containing 100 mM Tris, 50 mM EDTA, pH 7.4. The cells are dispersed to a homogeneous suspension using a high shear mixer.

5           The cells are then lysed by passing the solution through a microfluidizer (Microfluidics, Corp. or APV Gaulin, Inc.) twice at 4000-6000 psi. The homogenate is then mixed with NaCl solution to a final concentration of 0.5 M NaCl, followed by centrifugation at 7000 xg for 15 min. The resultant pellet is washed again using 0.5M NaCl, 100 mM Tris, 50 mM EDTA, pH 7.4.

10           The resulting washed inclusion bodies are solubilized with 1.5 M guanidine hydrochloride (GuHCl) for 2-4 hours. After 7000 xg centrifugation for 15 min., the pellet is discarded and the polypeptide containing supernatant is incubated at 4 degree C overnight to allow further GuHCl extraction.

          Following high speed centrifugation (30,000 xg) to remove insoluble particles,  
15           the GuHCl solubilized protein is refolded by quickly mixing the GuHCl extract with 20 volumes of buffer containing 50 mM sodium, pH 4.5, 150 mM NaCl, 2 mM EDTA by vigorous stirring. The refolded diluted protein solution is kept at 4 degree C without mixing for 12 hours prior to further purification steps.

          To clarify the refolded polypeptide solution, a previously prepared tangential  
20           filtration unit equipped with 0.16 um membrane filter with appropriate surface area (e.g., Filtron), equilibrated with 40 mM sodium acetate, pH 6.0 is employed. The filtered sample is loaded onto a cation exchange resin (e.g., Poros HS-50, Perseptive Biosystems). The column is washed with 40 mM sodium acetate, pH 6.0 and eluted with 250 mM, 500 mM, 1000 mM, and 1500 mM NaCl in the same buffer, in a  
25           stepwise manner. The absorbance at 280 nm of the effluent is continuously monitored. Fractions are collected and further analyzed by SDS-PAGE.



Fractions containing the polypeptide are then pooled and mixed with 4 volumes of water. The diluted sample is then loaded onto a previously prepared set of tandem columns of strong anion (Poros HQ-50, Perseptive Biosystems) and weak anion (Poros CM-20, Perseptive Biosystems) exchange resins. The columns are  
5 equilibrated with 40 mM sodium acetate, pH 6.0. Both columns are washed with 40 mM sodium acetate, pH 6.0, 200 mM NaCl. The CM-20 column is then eluted using a 10 column volume linear gradient ranging from 0.2 M NaCl, 50 mM sodium acetate, pH 6.0 to 1.0 M NaCl, 50 mM sodium acetate, pH 6.5. Fractions are collected under constant  $A_{280}$  monitoring of the effluent. Fractions containing the  
10 polypeptide (determined, for instance, by 16% SDS-PAGE) are then pooled.

The resultant polypeptide should exhibit greater than 95% purity after the above refolding and purification steps. No major contaminant bands should be observed from Commassie blue stained 16% SDS-PAGE gel when 5 ug of purified protein is loaded. The purified protein can also be tested for endotoxin/LPS  
15 contamination, and typically the LPS content is less than 0.1 ng/ml according to LAL assays.

#### **Example 7: Cloning and Expression of a Polypeptide in a Baculovirus**

##### **Expression System**

20 In this example, the plasmid shuttle vector pA2 is used to insert a polynucleotide into a baculovirus to express a polypeptide. This expression vector contains the strong polyhedrin promoter of the *Autographa californica* nuclear polyhedrosis virus (AcMNPV) followed by convenient restriction sites such as BamHI, Xba I and Asp718. The polyadenylation site of the simian virus 40 ("SV40")  
25 is used for efficient polyadenylation. For easy selection of recombinant virus, the plasmid contains the beta-galactosidase gene from *E. coli* under control of a weak

Drosophila promoter in the same orientation, followed by the polyadenylation signal of the polyhedrin gene. The inserted genes are flanked on both sides by viral sequences for cell-mediated homologous recombination with wild-type viral DNA to generate a viable virus that express the cloned polynucleotide.

5 Many other baculovirus vectors can be used in place of the vector above, such as pAc373, pVL941, and pAcIM1, as one skilled in the art would readily appreciate, as long as the construct provides appropriately located signals for transcription, translation, secretion and the like, including a signal peptide and an in-frame AUG as required. Such vectors are described, for instance, in Luckow et al., Virology 170:31-  
10 39 (1989).

Specifically, the cDNA sequence contained in the deposited clone, including the AUG initiation codon and the naturally associated leader sequence identified in Table 1, is amplified using the PCR protocol described in Example 1. If the naturally occurring signal sequence is used to produce the secreted protein, the pA2 vector does  
15 not need a second signal peptide. Alternatively, the vector can be modified (pA2 GP) to include a baculovirus leader sequence, using the standard methods described in Summers et al., "A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures," Texas Agricultural Experimental Station Bulletin No. 1555 (1987).

20 The amplified fragment is isolated from a 1% agarose gel using a commercially available kit ("Geneclean," BIO 101 Inc., La Jolla, Ca.). The fragment then is digested with appropriate restriction enzymes and again purified on a 1% agarose gel.

The plasmid is digested with the corresponding restriction enzymes and  
25 optionally, can be dephosphorylated using calf intestinal phosphatase, using routine

procedures known in the art. The DNA is then isolated from a 1% agarose gel using a commercially available kit ("GeneClean" BIO 101 Inc., La Jolla, Ca.).

The fragment and the dephosphorylated plasmid are ligated together with T4 DNA ligase. *E. coli* HB101 or other suitable *E. coli* hosts such as XL-1 Blue  
5 (Stratagene Cloning Systems, La Jolla, CA) cells are transformed with the ligation mixture and spread on culture plates. Bacteria containing the plasmid are identified by digesting DNA from individual colonies and analyzing the digestion product by gel electrophoresis. The sequence of the cloned fragment is confirmed by DNA sequencing.

10 Five ug of a plasmid containing the polynucleotide is co-transfected with 1.0 ug of a commercially available linearized baculovirus DNA ("BaculoGold™ baculovirus DNA", Pharmingen, San Diego, CA), using the lipofection method described by Felgner et al., Proc. Natl. Acad. Sci. USA 84:7413-7417 (1987). One ug of BaculoGold™ virus DNA and 5 ug of the plasmid are mixed in a sterile well of a  
15 microtiter plate containing 50 ul of serum-free Grace's medium (Life Technologies Inc., Gaithersburg, MD). Afterwards, 10 ul Lipofectin plus 90 ul Grace's medium are added, mixed and incubated for 15 minutes at room temperature. Then the transfection mixture is added drop-wise to Sf9 insect cells (ATCC CRL 1711) seeded in a 35 mm tissue culture plate with 1 ml Grace's medium without serum. The plate is  
20 then incubated for 5 hours at 27 degrees C. The transfection solution is then removed from the plate and 1 ml of Grace's insect medium supplemented with 10% fetal calf serum is added. Cultivation is then continued at 27 degrees C for four days.

After four days the supernatant is collected and a plaque assay is performed, as described by Summers and Smith, *supra*. An agarose gel with "Blue Gal" (Life  
25 Technologies Inc., Gaithersburg) is used to allow easy identification and isolation of gal-expressing clones, which produce blue-stained plaques. (A detailed description of

a "plaque assay" of this type can also be found in the user's guide for insect cell culture and baculovirology distributed by Life Technologies Inc., Gaithersburg, page 9-10.) After appropriate incubation, blue stained plaques are picked with the tip of a micropipettor (e.g., Eppendorf). The agar containing the recombinant viruses is then resuspended in a microcentrifuge tube containing 200 ul of Grace's medium and the suspension containing the recombinant baculovirus is used to infect Sf9 cells seeded in 35 mm dishes. Four days later the supernatants of these culture dishes are harvested and then they are stored at 4 degree C.

To verify the expression of the polypeptide, Sf9 cells are grown in Grace's medium supplemented with 10% heat-inactivated FBS. The cells are infected with the recombinant baculovirus containing the polynucleotide at a multiplicity of infection ("MOI") of about 2. If radiolabeled proteins are desired, 6 hours later the medium is removed and is replaced with SF900 II medium minus methionine and cysteine (available from Life Technologies Inc., Rockville, MD). After 42 hours, 5 uCi of <sup>35</sup>S-methionine and 5 uCi <sup>35</sup>S-cysteine (available from Amersham) are added. The cells are further incubated for 16 hours and then are harvested by centrifugation. The proteins in the supernatant as well as the intracellular proteins are analyzed by SDS-PAGE followed by autoradiography (if radiolabeled).

Microsequencing of the amino acid sequence of the amino terminus of purified protein may be used to determine the amino terminal sequence of the produced protein.

#### **Example 8: Expression of a Polypeptide in Mammalian Cells**

The polypeptide of the present invention can be expressed in a mammalian cell. A typical mammalian expression vector contains a promoter element, which mediates the initiation of transcription of mRNA, a protein coding sequence, and signals required for the termination of transcription and polyadenylation of the

transcript. Additional elements include enhancers, Kozak sequences and intervening sequences flanked by donor and acceptor sites for RNA splicing. Highly efficient transcription is achieved with the early and late promoters from SV40, the long terminal repeats (LTRs) from Retroviruses, e.g., RSV, HTLV1, HIV1 and the early promoter of the cytomegalovirus (CMV). However, cellular elements can also be  
5 used (e.g., the human actin promoter).

Suitable expression vectors for use in practicing the present invention include, for example, vectors such as pSVL and pMSG (Pharmacia, Uppsala, Sweden), pRSVcat (ATCC 37152), pSV2dhfr (ATCC 37146), pBC12MI (ATCC 67109),  
10 pCMVSPORT 2.0, and pCMVSPORT 3.0. Mammalian host cells that could be used include, human Hela, 293, H9 and Jurkat cells, mouse NIH3T3 and C127 cells, Cos 1, Cos 7 and CV1, quail QC1-3 cells, mouse L cells and Chinese hamster ovary (CHO) cells.

Alternatively, the polypeptide can be expressed in stable cell lines containing  
15 the polynucleotide integrated into a chromosome. The co-transfection with a selectable marker such as dhfr, gpt, neomycin, hygromycin allows the identification and isolation of the transfected cells.

The transfected gene can also be amplified to express large amounts of the encoded protein. The DHFR (dihydrofolate reductase) marker is useful in developing  
20 cell lines that carry several hundred or even several thousand copies of the gene of interest. (See, e.g., Alt, F. W., et al., J. Biol. Chem. 253:1357-1370 (1978); Hamlin, J. L. and Ma, C., Biochem. et Biophys. Acta, 1097:107-143 (1990); Page, M. J. and Sydenham, M. A., Biotechnology 9:64-68 (1991).) Another useful selection marker is the enzyme glutamine synthase (GS) (Murphy et al., Biochem J. 227:277-279  
25 (1991); Bebbington et al., Bio/Technology 10:169-175 (1992). Using these markers, the mammalian cells are grown in selective medium and the cells with the highest

resistance are selected. These cell lines contain the amplified gene(s) integrated into a chromosome. Chinese hamster ovary (CHO) and NSO cells are often used for the production of proteins.

Derivatives of the plasmid pSV2-dhfr (ATCC Accession No. 37146), the  
5 expression vectors pC4 (ATCC Accession No. 209646) and pC6 (ATCC Accession No. 209647) contain the strong promoter (LTR) of the Rous Sarcoma Virus (Cullen et al., Molecular and Cellular Biology, 438-447 (March, 1985)) plus a fragment of the CMV-enhancer (Boshart et al., Cell 41:521-530 (1985).) Multiple cloning sites, e.g., with the restriction enzyme cleavage sites BamHI, XbaI and Asp718, facilitate the  
10 cloning of the gene of interest. The vectors also contain the 3' intron, the polyadenylation and termination signal of the rat preproinsulin gene, and the mouse DHFR gene under control of the SV40 early promoter.

Specifically, the plasmid pC6, for example, is digested with appropriate restriction enzymes and then dephosphorylated using calf intestinal phosphates by  
15 procedures known in the art. The vector is then isolated from a 1% agarose gel.

A polynucleotide of the present invention is amplified according to the protocol outlined in Example 1. If the naturally occurring signal sequence is used to produce the secreted protein, the vector does not need a second signal peptide. Alternatively, if the naturally occurring signal sequence is not used, the vector can be  
20 modified to include a heterologous signal sequence. (See, e.g., WO 96/34891.)

The amplified fragment is isolated from a 1% agarose gel using a commercially available kit ("Geneclean," BIO 101 Inc., La Jolla, Ca.). The fragment then is digested with appropriate restriction enzymes and again purified on a 1% agarose gel.

25 The amplified fragment is then digested with the same restriction enzyme and purified on a 1% agarose gel. The isolated fragment and the dephosphorylated vector

are then ligated with T4 DNA ligase. *E. coli* HB101 or XL-1 Blue cells are then transformed and bacteria are identified that contain the fragment inserted into plasmid pC6 using, for instance, restriction enzyme analysis.

Chinese hamster ovary cells lacking an active DHFR gene is used for  
5 transfection. Five  $\mu$ g of the expression plasmid pC6 a pC4 is cotransfected with 0.5  
ug of the plasmid pSVneo using lipofectin (Felgner et al., *supra*). The plasmid pSV2-  
neo contains a dominant selectable marker, the *neo* gene from Tn5 encoding an  
enzyme that confers resistance to a group of antibiotics including G418. The cells are  
seeded in alpha minus MEM supplemented with 1 mg/ml G418. After 2 days, the  
10 cells are trypsinized and seeded in hybridoma cloning plates (Greiner, Germany) in  
alpha minus MEM supplemented with 10, 25, or 50 ng/ml of methotrexate plus 1  
mg/ml G418. After about 10-14 days single clones are trypsinized and then seeded in  
6-well petri dishes or 10 ml flasks using different concentrations of methotrexate (50  
nM, 100 nM, 200 nM, 400 nM, 800 nM). Clones growing at the highest  
15 concentrations of methotrexate are then transferred to new 6-well plates containing  
even higher concentrations of methotrexate (1  $\mu$ M, 2  $\mu$ M, 5  $\mu$ M, 10 mM, 20 mM).  
The same procedure is repeated until clones are obtained which grow at a  
concentration of 100 - 200  $\mu$ M. Expression of the desired gene product is analyzed,  
for instance, by SDS-PAGE and Western blot or by reversed phase HPLC analysis.

20

#### **Example 9: Protein Fusions**

The polypeptides of the present invention are preferably fused to other  
proteins. These fusion proteins can be used for a variety of applications. For  
example, fusion of the present polypeptides to His-tag, HA-tag, protein A, IgG  
25 domains, and maltose binding protein facilitates purification. (See Example 5; see  
also EP A 394,827; Traunecker, et al., Nature 331:84-86 (1988).) Similarly, fusion to

IgG-1, IgG-3, and albumin increases the half-life time in vivo. Nuclear localization signals fused to the polypeptides of the present invention can target the protein to a specific subcellular localization, while covalent heterodimer or homodimers can increase or decrease the activity of a fusion protein. Fusion proteins can also create  
5 chimeric molecules having more than one function. Finally, fusion proteins can increase solubility and/or stability of the fused protein compared to the non-fused protein. All of the types of fusion proteins described above can be made by modifying the following protocol, which outlines the fusion of a polypeptide to an IgG molecule, or the protocol described in Example 5.

10 Briefly, the human Fc portion of the IgG molecule can be PCR amplified, using primers that span the 5' and 3' ends of the sequence described below. These primers also should have convenient restriction enzyme sites that will facilitate cloning into an expression vector, preferably a mammalian expression vector.

For example, if pC4 (Accession No. 209646) is used, the human Fc portion  
15 can be ligated into the BamHI cloning site. Note that the 3' BamHI site should be destroyed. Next, the vector containing the human Fc portion is re-restricted with BamHI, linearizing the vector, and a polynucleotide of the present invention, isolated by the PCR protocol described in Example 1, is ligated into this BamHI site. Note that the polynucleotide is cloned without a stop codon, otherwise a fusion protein will  
20 not be produced.

If the naturally occurring signal sequence is used to produce the secreted protein, pC4 does not need a second signal peptide. Alternatively, if the naturally occurring signal sequence is not used, the vector can be modified to include a heterologous signal sequence. (See, e.g., WO 96/34891.)

25

Human IgG Fc region:



GGGATCCGGAGCCCAAATCTTCTGACAAAACTCACACATGCCCACCGTGC  
CCAGCACCTGAATTCGAGGGTGCACCGTCAGTCTTCCTCTTCCCCCAAAA  
CCCAAGGACACCCTCATGATCTCCCGGACTCCTGAGGTACATGCGTGGT  
GGTGGACGTAAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGG  
5 ACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTA  
CAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACT  
GGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCA  
ACCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAAC  
CACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAG  
10 GTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCAAGCGACATCGCCGT  
GGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCT  
CCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTG  
GACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCA  
TGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGG  
15 GTAAATGAGTGCGACGGCCGCGACTCTAGAGGAT (SEQ ID NO:1)

**Example 10: Production of an Antibody from a Polypeptide**

The antibodies of the present invention can be prepared by a variety of methods. (See, Current Protocols, Chapter 2.) As one example of such methods, cells  
20 expressing a polypeptide of the present invention is administered to an animal to induce the production of sera containing polyclonal antibodies. In a preferred method, a preparation of the secreted protein is prepared and purified to render it substantially free of natural contaminants. Such a preparation is then introduced into an animal in order to produce polyclonal antisera of greater specific activity.

25 In the most preferred method, the antibodies of the present invention are monoclonal antibodies (or protein binding fragments thereof). Such monoclonal

antibodies can be prepared using hybridoma technology. (Köhler et al., *Nature* 256:495 (1975); Köhler et al., *Eur. J. Immunol.* 6:511 (1976); Köhler et al., *Eur. J. Immunol.* 6:292 (1976); Hammerling et al., in: *Monoclonal Antibodies and T-Cell Hybridomas*, Elsevier, N.Y., pp. 563-681 (1981).) In general, such procedures

5 involve immunizing an animal (preferably a mouse) with polypeptide or, more preferably, with a secreted polypeptide-expressing cell. Such cells may be cultured in any suitable tissue culture medium; however, it is preferable to culture cells in Earle's modified Eagle's medium supplemented with 10% fetal bovine serum (inactivated at about 56 degrees C), and supplemented with about 10 g/l of nonessential amino acids,

10 about 1,000 U/ml of penicillin, and about 100 ug/ml of streptomycin.

The splenocytes of such mice are extracted and fused with a suitable myeloma cell line. Any suitable myeloma cell line may be employed in accordance with the present invention; however, it is preferable to employ the parent myeloma cell line (SP2O), available from the ATCC. After fusion, the resulting hybridoma cells are

15 selectively maintained in HAT medium, and then cloned by limiting dilution as described by Wands et al. (*Gastroenterology* 80:225-232 (1981).) The hybridoma cells obtained through such a selection are then assayed to identify clones which secrete antibodies capable of binding the polypeptide.

Alternatively, additional antibodies capable of binding to the polypeptide can

20 be produced in a two-step procedure using anti-idiotypic antibodies. Such a method makes use of the fact that antibodies are themselves antigens, and therefore, it is possible to obtain an antibody which binds to a second antibody. In accordance with this method, protein specific antibodies are used to immunize an animal, preferably a mouse. The splenocytes of such an animal are then used to produce hybridoma cells,

25 and the hybridoma cells are screened to identify clones which produce an antibody whose ability to bind to the protein-specific antibody can be blocked by the

polypeptide. Such antibodies comprise anti-idiotypic antibodies to the protein-specific antibody and can be used to immunize an animal to induce formation of further protein-specific antibodies.

It will be appreciated that Fab and F(ab')<sub>2</sub> and other fragments of the antibodies of the present invention may be used according to the methods disclosed herein. Such fragments are typically produced by proteolytic cleavage, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')<sub>2</sub> fragments). Alternatively, secreted protein-binding fragments can be produced through the application of recombinant DNA technology or through synthetic chemistry.

For in vivo use of antibodies in humans, it may be preferable to use "humanized" chimeric monoclonal antibodies. Such antibodies can be produced using genetic constructs derived from hybridoma cells producing the monoclonal antibodies described above. Methods for producing chimeric antibodies are known in the art. (See, for review, Morrison, Science 229:1202 (1985); Oi et al., BioTechniques 4:214 (1986); Cabilly et al., U.S. Patent No. 4,816,567; Taniguchi et al., EP 171496; Morrison et al., EP 173494; Neuberger et al., WO 8601533; Robinson et al., WO 8702671; Boulianne et al., Nature 312:643 (1984); Neuberger et al., Nature 314:268 (1985).)

#### **Example 11: Production Of Secreted Protein For High-Throughput Screening Assays**

The following protocol produces a supernatant containing a polypeptide to be tested. This supernatant can then be used in the Screening Assays described in

Examples 13-20.

First, dilute Poly-D-Lysine (644 587 Boehringer-Mannheim) stock solution (1mg/ml in PBS) 1:20 in PBS (w/o calcium or magnesium 17-516F Biowhittaker) for a working solution of 50ug/ml. Add 200 ul of this solution to each well (24 well plates) and incubate at RT for 20 minutes. Be sure to distribute the solution over each well (note: a 12-channel pipetter may be used with tips on every other channel). Aspirate off the Poly-D-Lysine solution and rinse with 1ml PBS (Phosphate Buffered Saline). The PBS should remain in the well until just prior to plating the cells and plates may be poly-lysine coated in advance for up to two weeks.

Plate 293T cells (do not carry cells past P+20) at  $2 \times 10^5$  cells/well in .5ml DMEM(Dulbecco's Modified Eagle Medium)(with 4.5 G/L glucose and L-glutamine (12-604F Biowhittaker))/10% heat inactivated FBS(14-503F Biowhittaker)/1x Penstrep(17-602E Biowhittaker). Let the cells grow overnight.

The next day, mix together in a sterile solution basin: 300 ul Lipofectamine (18324-012 Gibco/BRL) and 5ml Optimem I (31985070 Gibco/BRL)/96-well plate. With a small volume multi-channel pipetter, aliquot approximately 2ug of an expression vector containing a polynucleotide insert, produced by the methods described in Examples 8 or 9, into an appropriately labeled 96-well round bottom plate. With a multi-channel pipetter, add 50ul of the Lipofectamine/Optimem I mixture to each well. Pipette up and down gently to mix. Incubate at RT 15-45 minutes. After about 20 minutes, use a multi-channel pipetter to add 150ul Optimem I to each well. As a control, one plate of vector DNA lacking an insert should be transfected with each set of transfections.

Preferably, the transfection should be performed by tag-teaming the following tasks. By tag-teaming, hands on time is cut in half, and the cells do not spend too much time on PBS. First, person A aspirates off the media from four 24-well plates of cells, and then person B rinses each well with .5-1ml PBS. Person A then aspirates

off PBS rinse, and person B, using a 12-channel pipetter with tips on every other channel, adds the 200ul of DNA/Lipofectamine/Optimem I complex to the odd wells first, then to the even wells, to each row on the 24-well plates. Incubate at 37 degrees C for 6 hours.

- 5 While cells are incubating, prepare appropriate media, either 1%BSA in DMEM with 1x penstrep, or CHO-5 media (116.6 mg/L of  $\text{CaCl}_2$  (anhyd); 0.00130 mg/L  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ; 0.050 mg/L of  $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ ; 0.417 mg/L of  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ; 311.80 mg/L of Kcl; 28.64 mg/L of  $\text{MgCl}_2$ ; 48.84 mg/L of  $\text{MgSO}_4$ ; 6995.50 mg/L of NaCl; 2400.0 mg/L of  $\text{NaHCO}_3$ ; 62.50 mg/L of  $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ ; 71.02 mg/L of
- 10  $\text{Na}_2\text{HPO}_4$ ; .4320 mg/L of  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ ; .002 mg/L of Arachidonic Acid ; 1.022 mg/L of Cholesterol; .070 mg/L of DL-alpha-Tocopherol-Acetate; 0.0520 mg/L of Linoleic Acid; 0.010 mg/L of Linolenic Acid; 0.010 mg/L of Myristic Acid; 0.010 mg/L of Oleic Acid; 0.010 mg/L of Palmitic Acid; 0.010 mg/L of Palmitic Acid; 100 mg/L of Pluronic F-68; 0.010 mg/L of Stearic Acid; 2.20 mg/L of Tween 80; 4551 mg/L of D-
- 15 Glucose; 130.85 mg/ml of L- Alanine; 147.50 mg/ml of L-Arginine-HCL; 7.50 mg/ml of L-Asparagine- $\text{H}_2\text{O}$ ; 6.65 mg/ml of L-Aspartic Acid; 29.56 mg/ml of L-Cystine-2HCL- $\text{H}_2\text{O}$ ; 31.29 mg/ml of L-Cystine-2HCL; 7.35 mg/ml of L-Glutamic Acid; 365.0 mg/ml of L-Glutamine; 18.75 mg/ml of Glycine; 52.48 mg/ml of L-Histidine-HCL- $\text{H}_2\text{O}$ ; 106.97 mg/ml of L-Isoleucine; 111.45 mg/ml of L-Leucine; 163.75 mg/ml of L-
- 20 Lysine HCL; 32.34 mg/ml of L-Methionine; 68.48 mg/ml of L-Phenylalanine; 40.0 mg/ml of L-Proline; 26.25 mg/ml of L-Serine; 101.05 mg/ml of L-Threonine; 19.22 mg/ml of L-Tryptophan; 91.79 mg/ml of L-Tyrosine-2Na-2 $\text{H}_2\text{O}$ ; 99.65 mg/ml of L-Valine; 0.0035 mg/L of Biotin; 3.24 mg/L of D-Ca Pantothenate; 11.78 mg/L of Choline Chloride; 4.65 mg/L of Folic Acid; 15.60 mg/L of i-Inositol; 3.02 mg/L of
- 25 Niacinamide; 3.00 mg/L of Pyridoxal HCL; 0.031 mg/L of Pyridoxine HCL; 0.319 mg/L of Riboflavin; 3.17 mg/L of Thiamine HCL; 0.365 mg/L of Thymidine; and

0.680 mg/L of Vitamin B<sub>12</sub>; 25 mM of HEPES Buffer; 2.39 mg/L of Na Hypoxanthine; 0.105 mg/L of Lipoic Acid; 0.081 mg/L of Sodium Putrescine-2HCL; 55.0 mg/L of Sodium Pyruvate; 0.0067 mg/L of Sodium Selenite; 20uM of Ethanolamine; 0.122 mg/L of Ferric Citrate; 41.70 mg/L of Methyl-B-Cyclodextrin  
5 complexed with Linoleic Acid; 33.33 mg/L of Methyl-B-Cyclodextrin complexed with Oleic Acid; and 10 mg/L of Methyl-B-Cyclodextrin complexed with Retinal) with 2mm glutamine and 1x penstrep. (BSA (81-068-3 Bayer) 100gm dissolved in 1L DMEM for a 10% BSA stock solution). Filter the media and collect 50 ul for endotoxin assay in 15ml polystyrene conical.

- 10 The transfection reaction is terminated, preferably by tag-teaming, at the end of the incubation period. Person A aspirates off the transfection media, while person B adds 1.5ml appropriate media to each well. Incubate at 37 degrees C for 45 or 72 hours depending on the media used: 1%BSA for 45 hours or CHO-5 for 72 hours.

- On day four, using a 300ul multichannel pipetter, aliquot 600ul in one 1ml  
15 deep well plate and the remaining supernatant into a 2ml deep well. The supernatants from each well can then be used in the assays described in Examples 13-20.

- It is specifically understood that when activity is obtained in any of the assays described below using a supernatant, the activity originates from either the polypeptide directly (e.g., as a secreted protein) or by the polypeptide inducing  
20 expression of other proteins, which are then secreted into the supernatant. Thus, the invention further provides a method of identifying the protein in the supernatant characterized by an activity in a particular assay.

#### **Example 12: Construction of GAS Reporter Construct**

- 25 One signal transduction pathway involved in the differentiation and proliferation of cells is called the Jaks-STATs pathway. Activated proteins in the

Jaks-STATs pathway bind to gamma activation site "GAS" elements or interferon-sensitive responsive element ("ISRE"), located in the promoter of many genes. The binding of a protein to these elements alter the expression of the associated gene.

GAS and ISRE elements are recognized by a class of transcription factors called Signal Transducers and Activators of Transcription, or "STATs." There are six members of the STATs family. Stat1 and Stat3 are present in many cell types, as is Stat2 (as response to IFN-alpha is widespread). Stat4 is more restricted and is not in many cell types though it has been found in T helper class 1, cells after treatment with IL-12. Stat5 was originally called mammary growth factor, but has been found at higher concentrations in other cells including myeloid cells. It can be activated in tissue culture cells by many cytokines.

The STATs are activated to translocate from the cytoplasm to the nucleus upon tyrosine phosphorylation by a set of kinases known as the Janus Kinase ("Jaks") family. Jaks represent a distinct family of soluble tyrosine kinases and include Tyk2, Jak1, Jak2, and Jak3. These kinases display significant sequence similarity and are generally catalytically inactive in resting cells.

The Jaks are activated by a wide range of receptors summarized in the Table below. (Adapted from review by Schidler and Darnell, Ann. Rev. Biochem. 64:621-51 (1995).) A cytokine receptor family, capable of activating Jaks, is divided into two groups: (a) Class 1 includes receptors for IL-2, IL-3, IL-4, IL-6, IL-7, IL-9, IL-11, IL-12, IL-15, Epo, PRL, GH, G-CSF, GM-CSF, LIF, CNTF, and thrombopoietin; and (b) Class 2 includes IFN-a, IFN-g, and IL-10. The Class 1 receptors share a conserved cysteine motif (a set of four conserved cysteines and one tryptophan) and a WSXWS motif (a membrane proximal region encoding Trp-Ser-Xxx-Trp-Ser (SEQ ID NO:2)).

Thus, on binding of a ligand to a receptor, Jaks are activated, which in turn activate STATs, which then translocate and bind to GAS elements. This entire process is encompassed in the Jaks-STATs signal transduction pathway.

Therefore, activation of the Jaks-STATs pathway, reflected by the binding of  
5 the GAS or the ISRE element, can be used to indicate proteins involved in the proliferation and differentiation of cells. For example, growth factors and cytokines are known to activate the Jaks-STATs pathway. (See Table below.) Thus, by using GAS elements linked to reporter molecules, activators of the Jaks-STATs pathway can be identified.



	<u>Ligand</u>	<u>tyk2</u>	<u>JAKs</u> <u>Jak1</u>	<u>Jak2</u>	<u>Jak3</u>	<u>STATS</u>	<u>GAS(elements) or ISRE</u>
	<u>IFN family</u>						
5	IFN-a/B	+	+	-	-	1,2,3	ISRE
	IFN-g		+	+	-	1	GAS (IRF1>Lys6>IFP)
	Il-10	+	?	?	-	1,3	
	<u>gp130 family</u>						
10	IL-6 (Pleiotrophic)	+	+	+	?	1,3	GAS (IRF1>Lys6>IFP)
	Il-11(Pleiotrophic)	?	+	?	?	1,3	
	OnM(Pleiotrophic)	?	+	+	?	1,3	
	LIF(Pleiotrophic)	?	+	+	?	1,3	
	CNTF(Pleiotrophic)	-/+	+	+	?	1,3	
15	G-CSF(Pleiotrophic)	?	+	?	?	1,3	
	IL-12(Pleiotrophic)	+	-	+	+	1,3	
	<u>g-C family</u>						
	IL-2 (lymphocytes)	-	+	-	+	1,3,5	GAS
20	IL-4 (lymph/myeloid)	-	+	-	+	6	GAS (IRF1 = IFP >>Ly6)(IgH)
	IL-7 (lymphocytes)	-	+	-	+	5	GAS
	IL-9 (lymphocytes)	-	+	-	+	5	GAS
	IL-13 (lymphocyte)	-	+	?	?	6	GAS
	IL-15	?	+	?	+	5	GAS
25	<u>gp140 family</u>						
	IL-3 (myeloid)	-	-	+	-	5	GAS (IRF1>IFP>>Ly6)
	IL-5 (myeloid)	-	-	+	-	5	GAS
	GM-CSF (myeloid)	-	-	+	-	5	GAS
30	<u>Growth hormone family</u>						
	GH	?	-	+	-	5	
	PRL	?	+/-	+	-	1,3,5	
	EPO	?	-	+	-	5	GAS(B-CAS>IRF1=IFP>>Ly6)
35	<u>Receptor Tyrosine Kinases</u>						
	EGF	?	+	+	-	1,3	GAS (IRF1)
	PDGF	?	+	+	-	1,3	
	CSF-1	?	+	+	-	1,3	GAS (not IRF1)

To construct a synthetic GAS containing promoter element, which is used in the Biological Assays described in Examples 13-14, a PCR based strategy is employed to generate a GAS-SV40 promoter sequence. The 5' primer contains four tandem copies of the GAS binding site found in the IRF1 promoter and previously demonstrated to bind STATs upon induction with a range of cytokines (Rothman et al., Immunity 1:457-468 (1994).), although other GAS or ISRE elements can be used instead. The 5' primer also contains 18bp of sequence complementary to the SV40 early promoter sequence and is flanked with an XhoI site. The sequence of the 5' primer is:

10 5':GCGCCTCGAGATTTCCTCCCGAAATCTAGATTTCCTCCCGAAATGATTTCCTCCCGAAATGATTTCCTCCCGAAATATCTGCCATCTCAATTAG:3' (SEQ ID NO:3)

The downstream primer is complementary to the SV40 promoter and is flanked with a Hind III site: 5':GCGGCAAGCTTTTGTCAAAGCCTAGGC:3' (SEQ ID NO:4)

15 PCR amplification is performed using the SV40 promoter template present in the B-gal:promoter plasmid obtained from Clontech. The resulting PCR fragment is digested with XhoI/Hind III and subcloned into BLSK2-. (Stratagene.) Sequencing with forward and reverse primers confirms that the insert contains the following sequence:

20 5':CTCGAGATTTCCTCCCGAAATCTAGATTTCCTCCCGAAATGATTTCCTCCCGAAATGATTTCCTCCCGAAATATCTGCCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCTAACTCCGCCCATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTGACTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCGGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCT  
25 AGGCTTTTGCAAAAAGCTT:3' (SEQ ID NO:5)

With this GAS promoter element linked to the SV40 promoter, a GAS:SEAP2 reporter construct is next engineered. Here, the reporter molecule is a secreted alkaline phosphatase, or "SEAP." Clearly, however, any reporter molecule can be instead of SEAP, in this or in any of the other Examples. Well known reporter  
5 molecules that can be used instead of SEAP include chloramphenicol acetyltransferase (CAT), luciferase, alkaline phosphatase, B-galactosidase, green fluorescent protein (GFP), or any protein detectable by an antibody.

The above sequence confirmed synthetic GAS-SV40 promoter element is subcloned into the pSEAP-Promoter vector obtained from Clontech using HindIII and  
10 XhoI, effectively replacing the SV40 promoter with the amplified GAS:SV40 promoter element, to create the GAS-SEAP vector. However, this vector does not contain a neomycin resistance gene, and therefore, is not preferred for mammalian expression systems.

Thus, in order to generate mammalian stable cell lines expressing the GAS-  
15 SEAP reporter, the GAS-SEAP cassette is removed from the GAS-SEAP vector using SalI and NotI, and inserted into a backbone vector containing the neomycin resistance gene, such as pGFP-1 (Clontech), using these restriction sites in the multiple cloning site, to create the GAS-SEAP/Neo vector. Once this vector is transfected into mammalian cells, this vector can then be used as a reporter molecule for GAS binding  
20 as described in Examples 13-14.

Other constructs can be made using the above description and replacing GAS with a different promoter sequence. For example, construction of reporter molecules containing NFK-B and EGR promoter sequences are described in Examples 15 and  
16. However, many other promoters can be substituted using the protocols described  
25 in these Examples. For instance, SRE, IL-2, NFAT, or Osteocalcin promoters can be substituted, alone or in combination (e.g., GAS/NF-KB/EGR, GAS/NF-KB, IL-

2/NFAT, or NF-KB/GAS). Similarly, other cell lines can be used to test reporter construct activity, such as HELA (epithelial), HUVEC (endothelial), Reh (B-cell), Saos-2 (osteoblast), HUVAC (aortic), or Cardiomyocyte.

5 **Example 13: High-Throughput Screening Assay for T-cell Activity.**

The following protocol is used to assess T-cell activity by identifying factors, and determining whether supernate containing a polypeptide of the invention proliferates and/or differentiates T-cells. T-cell activity is assessed using the GAS/SEAP/Neo construct produced in Example 12. Thus, factors that increase SEAP  
10 activity indicate the ability to activate the Jaks-STATS signal transduction pathway. The T-cell used in this assay is Jurkat T-cells (ATCC Accession No. TIB-152), although Molt-3 cells (ATCC Accession No. CRL-1552) and Molt-4 cells (ATCC Accession No. CRL-1582) cells can also be used.

Jurkat T-cells are lymphoblastic CD4+ Th1 helper cells. In order to generate  
15 stable cell lines, approximately 2 million Jurkat cells are transfected with the GAS-SEAP/neo vector using DMRIE-C (Life Technologies)(transfection procedure described below). The transfected cells are seeded to a density of approximately 20,000 cells per well and transfectants resistant to 1 mg/ml gentamicin selected. Resistant colonies are expanded and then tested for their response to increasing  
20 concentrations of interferon gamma. The dose response of a selected clone is demonstrated.

Specifically, the following protocol will yield sufficient cells for 75 wells containing 200 ul of cells. Thus, it is either scaled up, or performed in multiple to generate sufficient cells for multiple 96 well plates. Jurkat cells are maintained in  
25 RPMI + 10% serum with 1% Pen-Strep. Combine 2.5 mls of OPTI-MEM (Life

Technologies) with 10 ug of plasmid DNA in a T25 flask. Add 2.5 ml OPTI-MEM containing 50 ul of DMRIE-C and incubate at room temperature for 15-45 mins.

During the incubation period, count cell concentration, spin down the required number of cells ( $10^7$  per transfection), and resuspend in OPTI-MEM to a final  
5 concentration of  $10^7$  cells/ml. Then add 1ml of  $1 \times 10^7$  cells in OPTI-MEM to T25 flask and incubate at 37 degrees C for 6 hrs. After the incubation, add 10 ml of RPMI + 15% serum.

The Jurkat:GAS-SEAP stable reporter lines are maintained in RPMI + 10% serum, 1 mg/ml Genticin, and 1% Pen-Strep. These cells are treated with  
10 supernatants containing polypeptides of the invention and/or induced polypeptides of the invention as produced by the protocol described in Example 11.

On the day of treatment with the supernatant, the cells should be washed and resuspended in fresh RPMI + 10% serum to a density of 500,000 cells per ml. The exact number of cells required will depend on the number of supernatants being  
15 screened. For one 96 well plate, approximately 10 million cells (for 10 plates, 100 million cells) are required.

Transfer the cells to a triangular reservoir boat, in order to dispense the cells into a 96 well dish, using a 12 channel pipette. Using a 12 channel pipette, transfer 200 ul of cells into each well (therefore adding 100, 000 cells per well).

20 After all the plates have been seeded, 50 ul of the supernatants are transferred directly from the 96 well plate containing the supernatants into each well using a 12 channel pipette. In addition, a dose of exogenous interferon gamma (0.1, 1.0, 10 ng) is added to wells H9, H10, and H11 to serve as additional positive controls for the assay.

25 The 96 well dishes containing Jurkat cells treated with supernatants are placed in an incubator for 48 hrs (note: this time is variable between 48-72 hrs). 35 ul

samples from each well are then transferred to an opaque 96 well plate using a 12 channel pipette. The opaque plates should be covered (using sellophene covers) and stored at -20 degrees C until SEAP assays are performed according to Example 17. The plates containing the remaining treated cells are placed at 4 degrees C and serve  
5 as a source of material for repeating the assay on a specific well if desired.

As a positive control, 100 Unit/ml interferon gamma can be used which is known to activate Jurkat T cells. Over 30 fold induction is typically observed in the positive control wells.

The above protocol may be used in the generation of both transient, as well as,  
10 stable transfected cells, which would be apparent to those of skill in the art.

#### **Example 14: High-Throughput Screening Assay Identifying Myeloid Activity**

The following protocol is used to assess myeloid activity by determining whether polypeptides of the invention proliferates and/or differentiates myeloid cells.

15 Myeloid cell activity is assessed using the GAS/SEAP/Neo construct produced in Example 12. Thus, factors that increase SEAP activity indicate the ability to activate the Jaks-STATS signal transduction pathway. The myeloid cell used in this assay is U937, a pre-monocyte cell line, although TF-1, HL60, or KG1 can be used.

To transiently transfect U937 cells with the GAS/SEAP/Neo construct  
20 produced in Example 12, a DEAE-Dextran method (Kharbanda et. al., 1994, Cell Growth & Differentiation, 5:259-265) is used. First, harvest  $2 \times 10^7$  U937 cells and wash with PBS. The U937 cells are usually grown in RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum (FBS) supplemented with 100 units/ml penicillin and 100 mg/ml streptomycin.

25 Next, suspend the cells in 1 ml of 20 mM Tris-HCl (pH 7.4) buffer containing 0.5 mg/ml DEAE-Dextran, 8 ug GAS-SEAP2 plasmid DNA, 140 mM NaCl, 5 mM

KCl, 375 uM Na<sub>2</sub>HPO<sub>4</sub>·7H<sub>2</sub>O, 1 mM MgCl<sub>2</sub>, and 675 uM CaCl<sub>2</sub>. Incubate at 37 degrees C for 45 min.

Wash the cells with RPMI 1640 medium containing 10% FBS and then resuspend in 10 ml complete medium and incubate at 37 degrees C for 36 hr.

- 5           The GAS-SEAP/U937 stable cells are obtained by growing the cells in 400 ug/ml G418. The G418-free medium is used for routine growth but every one to two months, the cells should be re-grown in 400 ug/ml G418 for couple of passages.

- These cells are tested by harvesting  $1 \times 10^8$  cells (this is enough for ten 96-well plates assay) and wash with PBS. Suspend the cells in 200 ml above described  
10       growth medium, with a final density of  $5 \times 10^5$  cells/ml. Plate 200 ul cells per well in the 96-well plate (or  $1 \times 10^5$  cells/well).

- Add 50 ul of the supernatant prepared by the protocol described in Example  
11. Incubate at 37 degrees C for 48 to 72 hr. As a positive control, 100 Unit/ml  
interferon gamma can be used which is known to activate U937 cells. Over 30 fold  
15       induction is typically observed in the positive control wells. SEAP assay the  
supernatant according to the protocol described in Example 17.

**Example 15: High-Throughput Screening Assay Identifying Neuronal Activity.**

- When cells undergo differentiation and proliferation, a group of genes are  
20       activated through many different signal transduction pathways. One of these genes,  
EGR1 (early growth response gene 1), is induced in various tissues and cell types  
upon activation. The promoter of EGR1 is responsible for such induction. Using the  
EGR1 promoter linked to reporter molecules, activation of cells can be assessed.

- Particularly, the following protocol is used to assess neuronal activity in PC12  
25       cell lines. PC12 cells (rat pheochromocytoma cells) are known to proliferate and/or  
differentiate by activation with a number of mitogens, such as TPA (tetradecanoyl

phorbol acetate), NGF (nerve growth factor), and EGF (epidermal growth factor).

The EGR1 gene expression is activated during this treatment. Thus, by stably transfecting PC12 cells with a construct containing an EGR promoter linked to SEAP reporter, activation of PC12 cells can be assessed.

- 5           The EGR/SEAP reporter construct can be assembled by the following protocol. The EGR-1 promoter sequence (-633 to +1)(Sakamoto K et al., Oncogene 6:867-871 (1991)) can be PCR amplified from human genomic DNA using the following primers:

5' GCGCTCGAGGGATGACAGCGATAGAACCCCGG -3' (SEQ ID NO:6)

- 10           5' GCGAAGCTTCGCGACTCCCCGGATCCGCCTC-3' (SEQ ID NO:7)

- Using the GAS:SEAP/Neo vector produced in Example 12, EGR1 amplified product can then be inserted into this vector. Linearize the GAS:SEAP/Neo vector using restriction enzymes XhoI/HindIII, removing the GAS/SV40 stuffer. Restrict the EGR1 amplified product with these same enzymes. Ligate the vector and the EGR1  
15 promoter.

          To prepare 96 well-plates for cell culture, two mls of a coating solution (1:30 dilution of collagen type I (Upstate Biotech Inc. Cat#08-115) in 30% ethanol (filter sterilized)) is added per one 10 cm plate or 50 ml per well of the 96-well plate, and allowed to air dry for 2 hr.

- 20           PC12 cells are routinely grown in RPMI-1640 medium (Bio Whittaker) containing 10% horse serum (JRH BIOSCIENCES, Cat. # 12449-78P), 5% heat-inactivated fetal bovine serum (FBS) supplemented with 100 units/ml penicillin and 100 ug/ml streptomycin on a precoated 10 cm tissue culture dish. One to four split is done every three to four days. Cells are removed from the plates by scraping and  
25 resuspended with pipetting up and down for more than 15 times.



Transfect the EGR/SEAP/Neo construct into PC12 using the Lipofectamine protocol described in Example 11. EGR-SEAP/PC12 stable cells are obtained by growing the cells in 300 ug/ml G418. The G418-free medium is used for routine growth but every one to two months, the cells should be re-grown in 300 ug/ml G418 for couple of passages.

To assay for neuronal activity, a 10 cm plate with cells around 70 to 80% confluent is screened by removing the old medium. Wash the cells once with PBS (Phosphate buffered saline). Then starve the cells in low serum medium (RPMI-1640 containing 1% horse serum and 0.5% FBS with antibiotics) overnight.

The next morning, remove the medium and wash the cells with PBS. Scrape off the cells from the plate, suspend the cells well in 2 ml low serum medium. Count the cell number and add more low serum medium to reach final cell density as  $5 \times 10^5$  cells/ml.

Add 200 ul of the cell suspension to each well of 96-well plate (equivalent to  $1 \times 10^5$  cells/well). Add 50 ul supernatant produced by Example 11, 37°C for 48 to 72 hr. As a positive control, a growth factor known to activate PC12 cells through EGR can be used, such as 50 ng/ul of Neuronal Growth Factor (NGF). Over fifty-fold induction of SEAP is typically seen in the positive control wells. SEAP assay the supernatant according to Example 17.

#### **Example 16: High-Throughput Screening Assay for T-cell Activity**

NF-KB (Nuclear Factor KB) is a transcription factor activated by a wide variety of agents including the inflammatory cytokines IL-1 and TNF, CD30 and CD40, lymphotoxin-alpha and lymphotoxin-beta, by exposure to LPS or thrombin, and by expression of certain viral gene products. As a transcription factor, NF-KB regulates the expression of genes involved in immune cell activation, control of

apoptosis (NF- $\kappa$ B appears to shield cells from apoptosis), B and T-cell development, anti-viral and antimicrobial responses, and multiple stress responses.

In non-stimulated conditions, NF- $\kappa$ B is retained in the cytoplasm with I- $\kappa$ B (Inhibitor  $\kappa$ B). However, upon stimulation, I- $\kappa$ B is phosphorylated and degraded,  
5 causing NF- $\kappa$ B to shuttle to the nucleus, thereby activating transcription of target genes. Target genes activated by NF- $\kappa$ B include IL-2, IL-6, GM-CSF, ICAM-1 and class I MHC.

Due to its central role and ability to respond to a range of stimuli, reporter constructs utilizing the NF- $\kappa$ B promoter element are used to screen the supernatants  
10 produced in Example 11. Activators or inhibitors of NF- $\kappa$ B would be useful in treating diseases. For example, inhibitors of NF- $\kappa$ B could be used to treat those diseases related to the acute or chronic activation of NF- $\kappa$ B, such as rheumatoid arthritis.

To construct a vector containing the NF- $\kappa$ B promoter element, a PCR based  
15 strategy is employed. The upstream primer contains four tandem copies of the NF- $\kappa$ B binding site (GGGGACTTTCCC) (SEQ ID NO:8), 18 bp of sequence complementary to the 5' end of the SV40 early promoter sequence, and is flanked with an XhoI site:

5':GCGGCCTCGAGGGGACTTTCCCGGGGACTTTCCGGGGACTTTCCGGGAC  
20 TTTCCATCCTGCCATCTCAATTAG:3' (SEQ ID NO:9)

The downstream primer is complementary to the 3' end of the SV40 promoter and is flanked with a Hind III site:

5':GCGGCAAGCTTTTGGCAAAGCCTAGGC:3' (SEQ ID NO:4)

PCR amplification is performed using the SV40 promoter template present in  
25 the pB-gal:promoter plasmid obtained from Clontech. The resulting PCR fragment is digested with XhoI and Hind III and subcloned into BLSK2-. (Stratagene)

Sequencing with the T7 and T3 primers confirms the insert contains the following sequence:

5':CTCGAGGGGACTTTCCCGGGGACTTTCCGGGGACTTTCCGGGGACTTTCC  
5 ATCTGCCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCTAACTCCGCCC  
ATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTGA  
CTAATTTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCGGCCTCTGAGCTA  
TTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAA  
GCTT:3' (SEQ ID NO:10)

10

Next, replace the SV40 minimal promoter element present in the pSEAP2-promoter plasmid (Clontech) with this NF-KB/SV40 fragment using XhoI and HindIII. However, this vector does not contain a neomycin resistance gene, and therefore, is not preferred for mammalian expression systems.

15

In order to generate stable mammalian cell lines, the NF-KB/SV40/SEAP cassette is removed from the above NF-KB/SEAP vector using restriction enzymes SalI and NotI, and inserted into a vector containing neomycin resistance. Particularly, the NF-KB/SV40/SEAP cassette was inserted into pGFP-1 (Clontech), replacing the GFP gene, after restricting pGFP-1 with SalI and NotI.

20

Once NF-KB/SV40/SEAP/Neo vector is created, stable Jurkat T-cells are created and maintained according to the protocol described in Example 13. Similarly, the method for assaying supernatants with these stable Jurkat T-cells is also described in Example 13. As a positive control, exogenous TNF alpha (0.1, 1, 10 ng) is added to wells H9, H10, and H11, with a 5-10 fold activation typically observed.

25

#### **Example 17: Assay for SEAP Activity**

As a reporter molecule for the assays described in Examples 13-16, SEAP activity is assayed using the Tropix Phospho-light Kit (Cat. BP-400) according to the following general procedure. The Tropix Phospho-light Kit supplies the Dilution, Assay, and Reaction Buffers used below.

- 5           Prime a dispenser with the 2.5x Dilution Buffer and dispense 15 ul of 2.5x dilution buffer into Optiplates containing 35 ul of a supernatant. Seal the plates with a plastic sealer and incubate at 65 degree C for 30 min. Separate the Optiplates to avoid uneven heating.

- 10           Cool the samples to room temperature for 15 minutes. Empty the dispenser and prime with the Assay Buffer. Add 50 ml Assay Buffer and incubate at room temperature 5 min. Empty the dispenser and prime with the Reaction Buffer (see the table below). Add 50 ul Reaction Buffer and incubate at room temperature for 20 minutes. Since the intensity of the chemiluminescent signal is time dependent, and it takes about 10 minutes to read 5 plates on luminometer, one should treat 5 plates at
- 15           each time and start the second set 10 minutes later.

Read the relative light unit in the luminometer. Set H12 as blank, and print the results. An increase in chemiluminescence indicates reporter activity.

**Reaction Buffer Formulation:**

# of plates	Rxn buffer diluent (ml)	CSPD (ml)
10	60	3
11	65	3.25
12	70	3.5
13	75	3.75
14	80	4
15	85	4.25
16	90	4.5
17	95	4.75
18	100	5
19	105	5.25
20	110	5.5
21	115	5.75
22	120	6

23	125	6.25
24	130	6.5
25	135	6.75
26	140	7
27	145	7.25
28	150	7.5
29	155	7.75
30	160	8
31	165	8.25
32	170	8.5
33	175	8.75
34	180	9
35	185	9.25
36	190	9.5
37	195	9.75
38	200	10
39	205	10.25
40	210	10.5
41	215	10.75
42	220	11
43	225	11.25
44	230	11.5
45	235	11.75
46	240	12
47	245	12.25
48	250	12.5
49	255	12.75
50	260	13

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**Example 18: High-Throughput Screening Assay Identifying Changes in Small Molecule Concentration and Membrane Permeability**

Binding of a ligand to a receptor is known to alter intracellular levels of small  
5 molecules, such as calcium, potassium, sodium, and pH, as well as alter membrane  
potential. These alterations can be measured in an assay to identify supernatants  
which bind to receptors of a particular cell. Although the following protocol  
describes an assay for calcium, this protocol can easily be modified to detect changes  
in potassium, sodium, pH, membrane potential, or any other small molecule which is  
10 detectable by a fluorescent probe.

The following assay uses Fluorometric Imaging Plate Reader ("FLIPR") to measure changes in fluorescent molecules (Molecular Probes) that bind small molecules. Clearly, any fluorescent molecule detecting a small molecule can be used instead of the calcium fluorescent molecule, fluo-4 (Molecular Probes, Inc.; catalog no. F-14202), used here.

For adherent cells, seed the cells at 10,000 -20,000 cells/well in a Co-star black 96-well plate with clear bottom. The plate is incubated in a CO<sub>2</sub> incubator for 20 hours. The adherent cells are washed two times in Biotek washer with 200 ul of HBSS (Hank's Balanced Salt Solution) leaving 100 ul of buffer after the final wash.

10 A stock solution of 1 mg/ml fluo-4 is made in 10% pluronic acid DMSO. To load the cells with fluo-4, 50 ul of 12 ug/ml fluo-4 is added to each well. The plate is incubated at 37 degrees C in a CO<sub>2</sub> incubator for 60 min. The plate is washed four times in the Biotek washer with HBSS leaving 100 ul of buffer.

For non-adherent cells, the cells are spun down from culture media. Cells are re-suspended to  $2-5 \times 10^6$  cells/ml with HBSS in a 50-ml conical tube. 4 ul of 1 mg/ml fluo-4 solution in 10% pluronic acid DMSO is added to each ml of cell suspension. The tube is then placed in a 37 degrees C water bath for 30-60 min. The cells are washed twice with HBSS, resuspended to  $1 \times 10^6$  cells/ml, and dispensed into a microplate, 100 ul/well. The plate is centrifuged at 1000 rpm for 5 min. The plate is

15

20 then washed once in Denley CellWash with 200 ul, followed by an aspiration step to 100 ul final volume.

For a non-cell based assay, each well contains a fluorescent molecule, such as fluo-4. The supernatant is added to the well, and a change in fluorescence is detected.

25 To measure the fluorescence of intracellular calcium, the FLIPR is set for the following parameters: (1) System gain is 300-800 mW; (2) Exposure time is 0.4

second; (3) Camera F/stop is F/2; (4) Excitation is 488 nm; (5) Emission is 530 nm; and (6) Sample addition is 50 ul. Increased emission at 530 nm indicates an extracellular signaling event which has resulted in an increase in the intracellular  $\text{Ca}^{++}$  concentration.

5

**Example 19: High-Throughput Screening Assay Identifying Tyrosine Kinase Activity**

The Protein Tyrosine Kinases (PTK) represent a diverse group of transmembrane and cytoplasmic kinases. Within the Receptor Protein Tyrosine Kinase (RPTK) group are receptors for a range of mitogenic and metabolic growth factors including the PDGF, FGF, EGF, NGF, HGF and Insulin receptor subfamilies. In addition there are a large family of RPTKs for which the corresponding ligand is unknown. Ligands for RPTKs include mainly secreted small proteins, but also membrane-bound and extracellular matrix proteins.

Activation of RPTK by ligands involves ligand-mediated receptor dimerization, resulting in transphosphorylation of the receptor subunits and activation of the cytoplasmic tyrosine kinases. The cytoplasmic tyrosine kinases include receptor associated tyrosine kinases of the src-family (e.g., src, yes, lck, lyn, fyn) and non-receptor linked and cytosolic protein tyrosine kinases, such as the Jak family, members of which mediate signal transduction triggered by the cytokine superfamily of receptors (e.g., the Interleukins, Interferons, GM-CSF, and Leptin).

Because of the wide range of known factors capable of stimulating tyrosine kinase activity, the identification of novel human secreted proteins capable of activating tyrosine kinase signal transduction pathways are of interest. Therefore, the following protocol is designed to identify those novel human secreted proteins capable of activating the tyrosine kinase signal transduction pathways.

Seed target cells (e.g., primary keratinocytes) at a density of approximately 25,000 cells per well in a 96 well Loprodyne Silent Screen Plates purchased from Nalge Nunc (Naperville, IL). The plates are sterilized with two 30 minute rinses with 100% ethanol, rinsed with water and dried overnight. Some plates are coated for 2 hr  
5 with 100 ml of cell culture grade type I collagen (50 mg/ml), gelatin (2%) or polylysine (50 mg/ml), all of which can be purchased from Sigma Chemicals (St. Louis, MO) or 10% Matrigel purchased from Becton Dickinson (Bedford, MA), or calf serum, rinsed with PBS and stored at 4 degree C. Cell growth on these plates is assayed by seeding 5,000 cells/well in growth medium and indirect quantitation of  
10 cell number through use of alamarBlue as described by the manufacturer Alamar Biosciences, Inc. (Sacramento, CA) after 48 hr. Falcon plate covers #3071 from Becton Dickinson (Bedford, MA) are used to cover the Loprodyne Silent Screen Plates. Falcon Microtest III cell culture plates can also be used in some proliferation experiments.

15 To prepare extracts, A431 cells are seeded onto the nylon membranes of Loprodyne plates (20,000/200ml/well) and cultured overnight in complete medium. Cells are quiesced by incubation in serum-free basal medium for 24 hr. After 5-20 minutes treatment with EGF (60ng/ml) or 50 ul of the supernatant produced in Example 11, the medium was removed and 100 ml of extraction buffer ((20 mM  
20 HEPES pH 7.5, 0.15 M NaCl, 1% Triton X-100, 0.1% SDS, 2 mM Na<sub>3</sub>VO<sub>4</sub>, 2 mM Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> and a cocktail of protease inhibitors (# 1836170) obtained from Boehringer Mannheim (Indianapolis, IN) is added to each well and the plate is shaken on a rotating shaker for 5 minutes at 4 degrees C. The plate is then placed in a vacuum transfer manifold and the extract filtered through the 0.45 mm membrane  
25 bottoms of each well using house vacuum. Extracts are collected in a 96-well catch/assay plate in the bottom of the vacuum manifold and immediately placed on



ice. To obtain extracts clarified by centrifugation, the content of each well, after detergent solubilization for 5 minutes, is removed and centrifuged for 15 minutes at 4 degrees C at 16,000 x g.

Test the filtered extracts for levels of tyrosine kinase activity. Although many  
5 methods of detecting tyrosine kinase activity are known, one method is described here.

Generally, the tyrosine kinase activity of a supernatant is evaluated by determining its ability to phosphorylate a tyrosine residue on a specific substrate (a biotinylated peptide). Biotinylated peptides that can be used for this purpose include  
10 PSK1 (corresponding to amino acids 6-20 of the cell division kinase cdc2-p34) and PSK2 (corresponding to amino acids 1-17 of gastrin). Both peptides are substrates for a range of tyrosine kinases and are available from Boehringer Mannheim.

The tyrosine kinase reaction is set up by adding the following components in order. First, add 10ul of 5uM Biotinylated Peptide, then 10ul ATP/Mg<sub>2+</sub> (5mM  
15 ATP/50mM MgCl<sub>2</sub>), then 10ul of 5x Assay Buffer (40mM imidazole hydrochloride, pH7.3, 40 mM beta-glycerophosphate, 1mM EGTA, 100mM MgCl<sub>2</sub>, 5 mM MnCl<sub>2</sub>, 0.5 mg/ml BSA), then 5ul of Sodium Vanadate(1mM), and then 5ul of water. Mix the components gently and preincubate the reaction mix at 30 degrees C for 2 min. Initial the reaction by adding 10ul of the control enzyme or the filtered supernatant.

20 The tyrosine kinase assay reaction is then terminated by adding 10 ul of 120mM EDTA and place the reactions on ice.

Tyrosine kinase activity is determined by transferring 50 ul aliquot of reaction mixture to a microtiter plate (MTP) module and incubating at 37 degrees C for 20 min. This allows the streptavidin coated 96 well plate to associate with the  
25 biotinylated peptide. Wash the MTP module with 300ul/well of PBS four times. Next add 75 ul of anti-phosphotyrosine antibody conjugated to horse radish

peroxidase(anti-P-Tyr-POD(0.5u/ml)) to each well and incubate at 37 degrees C for one hour. Wash the well as above.

- Next add 100ul of peroxidase substrate solution (Boehringer Mannheim) and incubate at room temperature for at least 5 mins (up to 30 min). Measure the
- 5 absorbance of the sample at 405 nm by using ELISA reader. The level of bound peroxidase activity is quantitated using an ELISA reader and reflects the level of tyrosine kinase activity.

**Example 20: High-Throughput Screening Assay Identifying Phosphorylation**

10 **Activity**

- As a potential alternative and/or compliment to the assay of protein tyrosine kinase activity described in Example 19, an assay which detects activation (phosphorylation) of major intracellular signal transduction intermediates can also be used. For example, as described below one particular assay can detect tyrosine
- 15 phosphorylation of the Erk-1 and Erk-2 kinases. However, phosphorylation of other molecules, such as Raf, JNK, p38 MAP, Map kinase kinase (MEK), MEK kinase, Src, Muscle specific kinase (MuSK), IRAK, Tec, and Janus, as well as any other phosphoserine, phosphotyrosine, or phosphothreonine molecule, can be detected by substituting these molecules for Erk-1 or Erk-2 in the following assay.

- 20 Specifically, assay plates are made by coating the wells of a 96-well ELISA plate with 0.1ml of protein G (1ug/ml) for 2 hr at room temp, (RT). The plates are then rinsed with PBS and blocked with 3% BSA/PBS for 1 hr at RT. The protein G plates are then treated with 2 commercial monoclonal antibodies (100ng/well) against Erk-1 and Erk-2 (1 hr at RT) (Santa Cruz Biotechnology). (To detect other molecules,
- 25 this step can easily be modified by substituting a monoclonal antibody detecting any

of the above described molecules.) After 3-5 rinses with PBS, the plates are stored at 4 degrees C until use.

A431 cells are seeded at 20,000/well in a 96-well Loprodyne filterplate and cultured overnight in growth medium. The cells are then starved for 48 hr in basal medium (DMEM) and then treated with EGF (6ng/well) or 50 ul of the supernatants obtained in Example 11 for 5-20 minutes. The cells are then solubilized and extracts filtered directly into the assay plate.

After incubation with the extract for 1 hr at RT, the wells are again rinsed. As a positive control, a commercial preparation of MAP kinase (10ng/well) is used in place of A431 extract. Plates are then treated with a commercial polyclonal (rabbit) antibody (1ug/ml) which specifically recognizes the phosphorylated epitope of the Erk-1 and Erk-2 kinases (1 hr at RT). This antibody is biotinylated by standard procedures. The bound polyclonal antibody is then quantitated by successive incubations with Europium-streptavidin and Europium fluorescence enhancing reagent in the Wallac DELFIA instrument (time-resolved fluorescence). An increased fluorescent signal over background indicates a phosphorylation.

**Example 21: Method of Determining Alterations in a Gene Corresponding to a Polynucleotide**

RNA isolated from entire families or individual patients presenting with a phenotype of interest (such as a disease) is be isolated. cDNA is then generated from these RNA samples using protocols known in the art. (See, Sambrook.) The cDNA is then used as a template for PCR, employing primers surrounding regions of interest in SEQ ID NO:X. Suggested PCR conditions consist of 35 cycles at 95 degrees C for 30 seconds; 60-120 seconds at 52-58 degrees C; and 60-120 seconds at 70 degrees C, using buffer solutions described in Sidransky et al., Science 252:706 (1991).

PCR products are then sequenced using primers labeled at their 5' end with T4 polynucleotide kinase, employing SequiTherm Polymerase. (Epicentre Technologies). The intron-exon borders of selected exons is also determined and genomic PCR products analyzed to confirm the results. PCR products harboring suspected mutations is then cloned and sequenced to validate the results of the direct sequencing.

PCR products is cloned into T-tailed vectors as described in Holton et al., Nucleic Acids Research, 19:1156 (1991) and sequenced with T7 polymerase (United States Biochemical). Affected individuals are identified by mutations not present in unaffected individuals.

Genomic rearrangements are also observed as a method of determining alterations in a gene corresponding to a polynucleotide. Genomic clones isolated according to Example 2 are nick-translated with digoxigenindeoxy-uridine 5'-triphosphate (Boehringer Mannheim), and FISH performed as described in Johnson et al., Methods Cell Biol. 35:73-99 (1991). Hybridization with the labeled probe is carried out using a vast excess of human cot-1 DNA for specific hybridization to the corresponding genomic locus.

Chromosomes are counterstained with 4,6-diamino-2-phenylidole and propidium iodide, producing a combination of C- and R-bands. Aligned images for precise mapping are obtained using a triple-band filter set (Chroma Technology, Brattleboro, VT) in combination with a cooled charge-coupled device camera (Photometrics, Tucson, AZ) and variable excitation wavelength filters. (Johnson et al., Genet. Anal. Tech. Appl., 8:75 (1991).) Image collection, analysis and chromosomal fractional length measurements are performed using the ISee Graphical Program System. (Inovision Corporation, Durham, NC.) Chromosome alterations of the genomic region hybridized by the probe are identified as insertions, deletions, and

translocations. These alterations are used as a diagnostic marker for an associated disease.

**Example 22: Method of Detecting Abnormal Levels of a Polypeptide in a**

5 **Biological Sample**

A polypeptide of the present invention can be detected in a biological sample, and if an increased or decreased level of the polypeptide is detected, this polypeptide is a marker for a particular phenotype. Methods of detection are numerous, and thus, it is understood that one skilled in the art can modify the following assay to fit their particular needs.

For example, antibody-sandwich ELISAs are used to detect polypeptides in a sample, preferably a biological sample. Wells of a microtiter plate are coated with specific antibodies, at a final concentration of 0.2 to 10 ug/ml. The antibodies are either monoclonal or polyclonal and are produced by the method described in Example 10. The wells are blocked so that non-specific binding of the polypeptide to the well is reduced.

The coated wells are then incubated for > 2 hours at RT with a sample containing the polypeptide. Preferably, serial dilutions of the sample should be used to validate results. The plates are then washed three times with deionized or distilled water to remove unbounded polypeptide.

Next, 50 ul of specific antibody-alkaline phosphatase conjugate, at a concentration of 25-400 ng, is added and incubated for 2 hours at room temperature. The plates are again washed three times with deionized or distilled water to remove unbounded conjugate.

25 Add 75 ul of 4-methylumbelliferyl phosphate (MUP) or p-nitrophenyl phosphate (NPP) substrate solution to each well and incubate 1 hour at room

temperature. Measure the reaction by a microtiter plate reader. Prepare a standard curve, using serial dilutions of a control sample, and plot polypeptide concentration on the X-axis (log scale) and fluorescence or absorbance of the Y-axis (linear scale). Interpolate the concentration of the polypeptide in the sample using the standard  
5 curve.

### **Example 23: Formulation**

The invention also provides methods of treatment and/or prevention diseases, disorders, and/or conditions (such as, for example, any one or more of the diseases or  
10 disorders disclosed herein) by administration to a subject of an effective amount of a Therapeutic. By therapeutic is meant a polynucleotides or polypeptides of the invention (including fragments and variants), agonists or antagonists thereof, and/or antibodies thereto, in combination with a pharmaceutically acceptable carrier type (e.g., a sterile carrier).

15 The Therapeutic will be formulated and dosed in a fashion consistent with good medical practice, taking into account the clinical condition of the individual patient (especially the side effects of treatment with the Therapeutic alone), the site of delivery, the method of administration, the scheduling of administration, and other factors known to practitioners. The "effective amount" for purposes herein is thus  
20 determined by such considerations.

As a general proposition, the total pharmaceutically effective amount of the Therapeutic administered parenterally per dose will be in the range of about 1 µg/kg/day to 10 mg/kg/day of patient body weight, although, as noted above, this will be subject to therapeutic discretion. More preferably, this dose is at least 0.01  
25 mg/kg/day, and most preferably for humans between about 0.01 and 1 mg/kg/day for the hormone. If given continuously, the Therapeutic is typically administered at a

dose rate of about 1 ug/kg/hour to about 50 ug/kg/hour, either by 1-4 injections per day or by continuous subcutaneous infusions, for example, using a mini-pump. An intravenous bag solution may also be employed. The length of treatment needed to observe changes and the interval following treatment for responses to occur appears to vary depending on the desired effect.

Therapeutics can be administered orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, gels, drops or transdermal patch), buccally, or as an oral or nasal spray. "Pharmaceutically acceptable carrier" refers to a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

Therapeutics of the invention are also suitably administered by sustained-release systems. Suitable examples of sustained-release Therapeutics are administered orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, gels, drops or transdermal patch), buccally, or as an oral or nasal spray. "Pharmaceutically acceptable carrier" refers to a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

Therapeutics of the invention are also suitably administered by sustained-release systems. Suitable examples of sustained-release Therapeutics include suitable polymeric materials (such as, for example, semi-permeable polymer matrices in the form of shaped articles, e.g., films, or microcapsules), suitable hydrophobic materials

(for example as an emulsion in an acceptable oil) or ion exchange resins, and sparingly soluble derivatives (such as, for example, a sparingly soluble salt).

Sustained-release matrices include polylactides (U.S. Pat. No. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate (Sidman et al.,  
5 Biopolymers 22:547-556 (1983)), poly (2- hydroxyethyl methacrylate) (Langer et al., J. Biomed. Mater. Res. 15:167-277 (1981), and Langer, Chem. Tech. 12:98-105 (1982)), ethylene vinyl acetate (Langer et al., Id.) or poly-D- (-)-3-hydroxybutyric acid (EP 133,988).

Sustained-release Therapeutics also include liposomally entrapped  
10 Therapeutics of the invention (*see generally*, Langer, *Science* 249:1527-1533 (1990); Treat et al., in *Liposomes in the Therapy of Infectious Disease and Cancer*, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 317 -327 and 353-365 (1989)). Liposomes containing the Therapeutic are prepared by methods known per se: DE 3,218,121; Epstein et al., Proc. Natl. Acad. Sci. (USA) 82:3688-3692 (1985); Hwang  
15 et al., Proc. Natl. Acad. Sci.(USA) 77:4030-4034 (1980); EP 52,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641; Japanese Pat. Appl. 83-118008; U.S. Pat. Nos. 4,485,045 and 4,544,545; and EP 102,324. Ordinarily, the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than about 30 mol. percent cholesterol, the selected proportion being adjusted for the  
20 optimal Therapeutic.

In yet an additional embodiment, the Therapeutics of the invention are delivered by way of a pump (*see Langer, supra*; Sefton, CRC Crit. Ref. Biomed. Eng. 14:201 (1987); Buchwald et al., Surgery 88:507 (1980); Saudek et al., N. Engl. J. Med. 321:574 (1989)).

25 Other controlled release systems are discussed in the review by Langer (*Science* 249:1527-1533 (1990)).



For parenteral administration, in one embodiment, the Therapeutic is formulated generally by mixing it at the desired degree of purity, in a unit dosage injectable form (solution, suspension, or emulsion), with a pharmaceutically acceptable carrier, i.e., one that is non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the formulation. For example, the formulation preferably does not include oxidizing agents and other compounds that are known to be deleterious to the Therapeutic.

Generally, the formulations are prepared by contacting the Therapeutic uniformly and intimately with liquid carriers or finely divided solid carriers or both. Then, if necessary, the product is shaped into the desired formulation. Preferably the carrier is a parenteral carrier, more preferably a solution that is isotonic with the blood of the recipient. Examples of such carrier vehicles include water, saline, Ringer's solution, and dextrose solution. Non-aqueous vehicles such as fixed oils and ethyl oleate are also useful herein, as well as liposomes.

The carrier suitably contains minor amounts of additives such as substances that enhance isotonicity and chemical stability. Such materials are non-toxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, succinate, acetic acid, and other organic acids or their salts; antioxidants such as ascorbic acid; low molecular weight (less than about ten residues) polypeptides, e.g., polyarginine or tripeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids, such as glycine, glutamic acid, aspartic acid, or arginine; monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, glucose, manose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; counterions such as sodium; and/or nonionic surfactants such as polysorbates, poloxamers, or PEG.

The Therapeutic is typically formulated in such vehicles at a concentration of about 0.1 mg/ml to 100 mg/ml, preferably 1-10 mg/ml, at a pH of about 3 to 8. It will be understood that the use of certain of the foregoing excipients, carriers, or stabilizers will result in the formation of polypeptide salts.

5           Any pharmaceutical used for therapeutic administration can be sterile. Sterility is readily accomplished by filtration through sterile filtration membranes (e.g., 0.2 micron membranes). Therapeutics generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

10           Therapeutics ordinarily will be stored in unit or multi-dose containers, for example, sealed ampoules or vials, as an aqueous solution or as a lyophilized formulation for reconstitution. As an example of a lyophilized formulation, 10-ml vials are filled with 5 ml of sterile-filtered 1% (w/v) aqueous Therapeutic solution, and the resulting mixture is lyophilized. The infusion solution is prepared by  
15           reconstituting the lyophilized Therapeutic using bacteriostatic Water-for-Injection.

          The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the Therapeutics of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or  
20           biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In addition, the Therapeutics may be employed in conjunction with other therapeutic compounds.

          The Therapeutics of the invention may be administered alone or in combination with adjuvants. Adjuvants that may be administered with the  
25           Therapeutics of the invention include, but are not limited to, alum, alum plus deoxycholate (ImmunoAg), MTP-PE (Biocine Corp.), QS21 (Genentech, Inc.), BCG,

and MPL. In a specific embodiment, Therapeutics of the invention are administered in combination with alum. In another specific embodiment, Therapeutics of the invention are administered in combination with QS-21. Further adjuvants that may be administered with the Therapeutics of the invention include, but are not limited to, Monophosphoryl lipid immunomodulator, AdjuVax 100a, QS-21, QS-18, CRL1005, Aluminum salts, MF-59, and Virosomal adjuvant technology. Vaccines that may be administered with the Therapeutics of the invention include, but are not limited to, vaccines directed toward protection against MMR (measles, mumps, rubella), polio, varicella, tetanus/diphtheria, hepatitis A, hepatitis B, haemophilus influenzae B, whooping cough, pneumonia, influenza, Lyme's Disease, rotavirus, cholera, yellow fever, Japanese encephalitis, poliomyelitis, rabies, typhoid fever, and pertussis. Combinations may be administered either concomitantly, e.g., as an admixture, separately but simultaneously or concurrently; or sequentially. This includes presentations in which the combined agents are administered together as a therapeutic mixture, and also procedures in which the combined agents are administered separately but simultaneously, e.g., as through separate intravenous lines into the same individual. Administration "in combination" further includes the separate administration of one of the compounds or agents given first, followed by the second.

The Therapeutics of the invention may be administered alone or in combination with other therapeutic agents. Therapeutic agents that may be administered in combination with the Therapeutics of the invention, include but not limited to, other members of the TNF family, chemotherapeutic agents, antibiotics, steroidal and non-steroidal anti-inflammatories, conventional immunotherapeutic agents, cytokines and/or growth factors. Combinations may be administered either concomitantly, e.g., as an admixture, separately but simultaneously or concurrently; or sequentially. This includes presentations in which the combined agents are

administered together as a therapeutic mixture, and also procedures in which the combined agents are administered separately but simultaneously, e.g., as through separate intravenous lines into the same individual. Administration "in combination" further includes the separate administration of one of the compounds or agents given first, followed by the second.

In one embodiment, the Therapeutics of the invention are administered in combination with members of the TNF family. TNF, TNF-related or TNF-like molecules that may be administered with the Therapeutics of the invention include, but are not limited to, soluble forms of TNF-alpha, lymphotoxin-alpha (LT-alpha, also known as TNF-beta), LT-beta (found in complex heterotrimer LT-alpha2-beta), OPGL, FasL, CD27L, CD30L, CD40L, 4-1BBL, DcR3, OX40L, TNF-gamma (International Publication No. WO 96/14328), AIM-I (International Publication No. WO 97/33899), endokine-alpha (International Publication No. WO 98/07880), TR6 (International Publication No. WO 98/30694), OPG, and neutrokine-alpha (International Publication No. WO 98/18921, OX40, and nerve growth factor (NGF), and soluble forms of Fas, CD30, CD27, CD40 and 4-IBB, TR2 (International Publication No. WO 96/34095), DR3 (International Publication No. WO 97/33904), DR4 (International Publication No. WO 98/32856), TR5 (International Publication No. WO 98/30693), TR6 (International Publication No. WO 98/30694), TR7 (International Publication No. WO 98/41629), TRANK, TR9 (International Publication No. WO 98/56892), TR10 (International Publication No. WO 98/54202), 312C2 (International Publication No. WO 98/06842), and TR12, and soluble forms CD154, CD70, and CD153.

In certain embodiments, Therapeutics of the invention are administered in combination with antiretroviral agents, nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and/or protease inhibitors.

Nucleoside reverse transcriptase inhibitors that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, RETROVIR™ (zidovudine/AZT), VIDEX™ (didanosine/ddI), HIVID™ (zalcitabine/ddC), ZERIT™ (stavudine/d4T), EPIVIR™ (lamivudine/3TC), and COMBIVIR™ (zidovudine/lamivudine). Non-nucleoside reverse transcriptase inhibitors that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, VIRAMUNE™ (nevirapine), RESCRIPTOR™ (delavirdine), and SUSTIVA™ (efavirenz). Protease inhibitors that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, CRIXIVAN™ (indinavir), NORVIR™ (ritonavir), INVIRASE™ (saquinavir), and VIRACEPT™ (nelfinavir). In a specific embodiment, antiretroviral agents, nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and/or protease inhibitors may be used in any combination with Therapeutics of the invention to treat AIDS and/or to prevent or treat HIV infection.

15 In other embodiments, Therapeutics of the invention may be administered in combination with anti-opportunistic infection agents. Anti-opportunistic agents that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, TRIMETHOPRIM-SULFAMETHOXAZOLE™, DAPSONE™, PENTAMIDINE™, ATOVAQUONE™, ISONIAZID™, 20 RIFAMPIN™, PYRAZINAMIDE™, ETHAMBUTOL™, RIFABUTIN™, CLARITHROMYCIN™, AZITHROMYCIN™, GANCICLOVIR™, FOSCARNET™, CIDOFOVIR™, FLUCONAZOLE™, ITRACONAZOLE™, KETOCONAZOLE™, ACYCLOVIR™, FAMCICOLVIR™, PYRIMETHAMINE™, LEUCOVORIN™, NEUPOGEN™ (filgrastim/G-CSF), and LEUKINE™ 25 (sargramostim/GM-CSF). In a specific embodiment, Therapeutics of the invention are used in any combination with TRIMETHOPRIM-SULFAMETHOXAZOLE™,

DAPSONE™, PENTAMIDINE™, and/or ATOVAQUONE™ to prophylactically treat or prevent an opportunistic *Pneumocystis carinii* pneumonia infection. In another specific embodiment, Therapeutics of the invention are used in any combination with ISONIAZID™, RIFAMPIN™, PYRAZINAMIDE™, and/or

5 ETHAMBUTOL™ to prophylactically treat or prevent an opportunistic *Mycobacterium avium* complex infection. In another specific embodiment, Therapeutics of the invention are used in any combination with RIFABUTIN™, CLARITHROMYCIN™, and/or AZITHROMYCIN™ to prophylactically treat or prevent an opportunistic *Mycobacterium tuberculosis* infection. In another specific

10 embodiment, Therapeutics of the invention are used in any combination with GANCICLOVIR™, FOSCARNET™, and/or CIDOFOVIR™ to prophylactically treat or prevent an opportunistic cytomegalovirus infection. In another specific embodiment, Therapeutics of the invention are used in any combination with FLUCONAZOLE™, ITRACONAZOLE™, and/or KETOCONAZOLE™ to

15 prophylactically treat or prevent an opportunistic fungal infection. In another specific embodiment, Therapeutics of the invention are used in any combination with ACYCLOVIR™ and/or FAMCICOLVIR™ to prophylactically treat or prevent an opportunistic herpes simplex virus type I and/or type II infection. In another specific embodiment, Therapeutics of the invention are used in any combination with

20 PYRIMETHAMINE™ and/or LEUCOVORIN™ to prophylactically treat or prevent an opportunistic *Toxoplasma gondii* infection. In another specific embodiment, Therapeutics of the invention are used in any combination with LEUCOVORIN™ and/or NEUPOGEN™ to prophylactically treat or prevent an opportunistic bacterial infection.

25 In a further embodiment, the Therapeutics of the invention are administered in combination with an antiviral agent. Antiviral agents that may be administered

with the Therapeutics of the invention include, but are not limited to, acyclovir, ribavirin, amantadine, and remantidine.

In a further embodiment, the Therapeutics of the invention are administered in combination with an antibiotic agent. Antibiotic agents that may be administered with the Therapeutics of the invention include, but are not limited to, amoxicillin, beta-lactamases, aminoglycosides, beta-lactam (glycopeptide), beta-lactamases, Clindamycin, chloramphenicol, cephalosporins, ciprofloxacin, ciprofloxacin, erythromycin, fluoroquinolones, macrolides, metronidazole, penicillins, quinolones, rifampin, streptomycin, sulfonamide, tetracyclines, trimethoprim, trimethoprim-sulfamthoxazole, and vancomycin.

Conventional nonspecific immunosuppressive agents, that may be administered in combination with the Therapeutics of the invention include, but are not limited to, steroids, cyclosporine, cyclosporine analogs, cyclophosphamide methylprednisone, prednisone, azathioprine, FK-506, 15-deoxyspergualin, and other immunosuppressive agents that act by suppressing the function of responding T cells.

In specific embodiments, Therapeutics of the invention are administered in combination with immunosuppressants. Immunosuppressants preparations that may be administered with the Therapeutics of the invention include, but are not limited to, ORTHOCLONE™ (OKT3), SANDIMMUNE™/NEORAL™/SANGDYA™ (cyclosporin), PROGRAF™ (tacrolimus), CELLCEPT™ (mycophenolate), Azathioprine, glucocorticosteroids, and RAPAMUNE™ (sirolimus). In a specific embodiment, immunosuppressants may be used to prevent rejection of organ or bone marrow transplantation.

In an additional embodiment, Therapeutics of the invention are administered alone or in combination with one or more intravenous immune globulin preparations. Intravenous immune globulin preparations that may be administered with the

Therapeutics of the invention include, but not limited to, GAMMAR™, IVEEGAM™, SANDOGLOBULIN™, GAMMAGARD S/D™, and GAMIMUNE™.

In a specific embodiment, Therapeutics of the invention are administered in combination with intravenous immune globulin preparations in transplantation therapy (e.g., bone marrow transplant).

In an additional embodiment, the Therapeutics of the invention are administered alone or in combination with an anti-inflammatory agent. Anti-inflammatory agents that may be administered with the Therapeutics of the invention include, but are not limited to, glucocorticoids and the nonsteroidal anti-inflammatories, aminoarylcarboxylic acid derivatives, arylacetic acid derivatives, arylbutyric acid derivatives, arylcarboxylic acids, arylpropionic acid derivatives, pyrazoles, pyrazolones, salicylic acid derivatives, thiazinecarboxamides, e-acetamidocaproic acid, S-adenosylmethionine, 3-amino-4-hydroxybutyric acid, amixetrine, bendazac, benzydamine, bucolome, difenpiramide, ditazol, emorfazone, guaiazulene, nabumetone, nimesulide, orgotein, oxaceprol, paranyline, perisoxal, pifoxime, proquazone, proxazole, and tenidap.

In another embodiment, compositions of the invention are administered in combination with a chemotherapeutic agent. Chemotherapeutic agents that may be administered with the Therapeutics of the invention include, but are not limited to, antibiotic derivatives (e.g., doxorubicin, bleomycin, daunorubicin, and dactinomycin); antiestrogens (e.g., tamoxifen); antimetabolites (e.g., fluorouracil, 5-FU, methotrexate, floxuridine, interferon alpha-2b, glutamic acid, plicamycin, mercaptopurine, and 6-thioguanine); cytotoxic agents (e.g., carmustine, BCNU, lomustine, CCNU, cytosine arabinoside, cyclophosphamide, estramustine, hydroxyurea, procarbazine, mitomycin, busulfan, cis-platin, and vincristine sulfate); hormones (e.g., medroxyprogesterone, estramustine phosphate sodium, ethinyl



estradiol, estradiol, megestrol acetate, methyltestosterone, diethylstilbestrol diphosphate, chlorotrianisene, and testolactone); nitrogen mustard derivatives (e.g., mephalen, chorambucil, mechlorethamine (nitrogen mustard) and thiotepa); steroids and combinations (e.g., bethamethasone sodium phosphate); and others (e.g.,  
5 dicarbazine, asparaginase, mitotane, vincristine sulfate, vinblastine sulfate, and etoposide).

In a specific embodiment, Therapeutics of the invention are administered in combination with CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) or any combination of the components of CHOP. In another  
10 embodiment, Therapeutics of the invention are administered in combination with Rituximab. In a further embodiment, Therapeutics of the invention are administered with Rituxmab and CHOP, or Rituxmab and any combination of the components of CHOP.

In an additional embodiment, the Therapeutics of the invention are  
15 administered in combination with cytokines. Cytokines that may be administered with the Therapeutics of the invention include, but are not limited to, IL2, IL3, IL4, IL5, IL6, IL7, IL10, IL12, IL13, IL15, anti-CD40, CD40L, IFN-gamma and TNF-alpha. In another embodiment, Therapeutics of the invention may be administered with any interleukin, including, but not limited to, IL-1alpha, IL-1beta, IL-2, IL-3,  
20 IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, and IL-21.

In an additional embodiment, the Therapeutics of the invention are administered in combination with angiogenic proteins. Angiogenic proteins that may be administered with the Therapeutics of the invention include, but are not limited to,  
25 Glioma Derived Growth Factor (GDGF), as disclosed in European Patent Number EP-399816; Platelet Derived Growth Factor-A (PDGF-A), as disclosed in European

Patent Number EP-682110; Platelet Derived Growth Factor-B (PDGF-B), as disclosed in European Patent Number EP-282317; Placental Growth Factor (PIGF), as disclosed in International Publication Number WO 92/06194; Placental Growth Factor-2 (PIGF-2), as disclosed in Hauser et al., Growth Factors, 4:259-268 (1993);

5 Vascular Endothelial Growth Factor (VEGF), as disclosed in International Publication Number WO 90/13649; Vascular Endothelial Growth Factor-A (VEGF-A), as disclosed in European Patent Number EP-506477; Vascular Endothelial Growth Factor-2 (VEGF-2), as disclosed in International Publication Number WO 96/39515; Vascular Endothelial Growth Factor B (VEGF-3); Vascular Endothelial Growth

10 Factor B-186 (VEGF-B186), as disclosed in International Publication Number WO 96/26736; Vascular Endothelial Growth Factor-D (VEGF-D), as disclosed in International Publication Number WO 98/02543; Vascular Endothelial Growth Factor-D (VEGF-D), as disclosed in International Publication Number WO 98/07832; and Vascular Endothelial Growth Factor-E (VEGF-E), as disclosed in German Patent

15 Number DE19639601. The above mentioned references are incorporated herein by reference herein.

In an additional embodiment, the Therapeutics of the invention are administered in combination with hematopoietic growth factors. Hematopoietic growth factors that may be administered with the Therapeutics of the invention

20 include, but are not limited to, LEUKINE™ (SARGRAMOSTIM™) and NEUPOGEN™ (FILGRASTIM™).

In an additional embodiment, the Therapeutics of the invention are administered in combination with Fibroblast Growth Factors. Fibroblast Growth Factors that may be administered with the Therapeutics of the invention include, but

25 are not limited to, FGF-1, FGF-2, FGF-3, FGF-4, FGF-5, FGF-6, FGF-7, FGF-8, FGF-9, FGF-10, FGF-11, FGF-12, FGF-13, FGF-14, and FGF-15.

In additional embodiments, the Therapeutics of the invention are administered in combination with other therapeutic or prophylactic regimens, such as, for example, radiation therapy.

5    **Example 24: Method of Treating Decreased Levels of the Polypeptide**

The present invention relates to a method for treating an individual in need of an increased level of a polypeptide of the invention in the body comprising administering to such an individual a composition comprising a therapeutically effective amount of an agonist of the invention (including polypeptides of the  
10    invention). Moreover, it will be appreciated that conditions caused by a decrease in the standard or normal expression level of a secreted protein in an individual can be treated by administering the polypeptide of the present invention, preferably in the secreted form. Thus, the invention also provides a method of treatment of an individual in need of an increased level of the polypeptide comprising administering  
15    to such an individual a Therapeutic comprising an amount of the polypeptide to increase the activity level of the polypeptide in such an individual.

For example, a patient with decreased levels of a polypeptide receives a daily dose 0.1-100 ug/kg of the polypeptide for six consecutive days. Preferably, the polypeptide is in the secreted form. The exact details of the dosing scheme, based on  
20    administration and formulation, are provided in Example 23.

**Example 25: Method of Treating Increased Levels of the Polypeptide**

The present invention also relates to a method of treating an individual in need of a decreased level of a polypeptide of the invention in the body comprising  
25    administering to such an individual a composition comprising a therapeutically

effective amount of an antagonist of the invention (including polypeptides and antibodies of the invention).

In one example, antisense technology is used to inhibit production of a polypeptide of the present invention. This technology is one example of a method of decreasing levels of a polypeptide, preferably a secreted form, due to a variety of etiologies, such as cancer. For example, a patient diagnosed with abnormally increased levels of a polypeptide is administered intravenously antisense polynucleotides at 0.5, 1.0, 1.5, 2.0 and 3.0 mg/kg day for 21 days. This treatment is repeated after a 7-day rest period if the treatment was well tolerated. The formulation of the antisense polynucleotide is provided in Example 23.

#### **Example 26: Method of Treatment Using Gene Therapy-Ex Vivo**

One method of gene therapy transplants fibroblasts, which are capable of expressing a polypeptide, onto a patient. Generally, fibroblasts are obtained from a subject by skin biopsy. The resulting tissue is placed in tissue-culture medium and separated into small pieces. Small chunks of the tissue are placed on a wet surface of a tissue culture flask, approximately ten pieces are placed in each flask. The flask is turned upside down, closed tight and left at room temperature over night. After 24 hours at room temperature, the flask is inverted and the chunks of tissue remain fixed to the bottom of the flask and fresh media (e.g., Ham's F12 media, with 10% FBS, penicillin and streptomycin) is added. The flasks are then incubated at 37 degree C for approximately one week.

At this time, fresh media is added and subsequently changed every several days. After an additional two weeks in culture, a monolayer of fibroblasts emerge. The monolayer is trypsinized and scaled into larger flasks.

pMV-7 (Kirschmeier, P.T. et al., DNA, 7:219-25 (1988)), flanked by the long terminal repeats of the Moloney murine sarcoma virus, is digested with EcoRI and HindIII and subsequently treated with calf intestinal phosphatase. The linear vector is fractionated on agarose gel and purified, using glass beads.

5           The cDNA encoding a polypeptide of the present invention can be amplified using PCR primers which correspond to the 5' and 3' end sequences respectively as set forth in Example 1 using primers and having appropriate restriction sites and initiation/stop codons, if necessary. Preferably, the 5' primer contains an EcoRI site and the 3' primer includes a HindIII site. Equal quantities of the Moloney murine  
10 sarcoma virus linear backbone and the amplified EcoRI and HindIII fragment are added together, in the presence of T4 DNA ligase. The resulting mixture is maintained under conditions appropriate for ligation of the two fragments. The ligation mixture is then used to transform bacteria HB101, which are then plated onto agar containing kanamycin for the purpose of confirming that the vector has the gene  
15 of interest properly inserted.

          The amphotropic pA317 or GP+am12 packaging cells are grown in tissue culture to confluent density in Dulbecco's Modified Eagles Medium (DMEM) with 10% calf serum (CS), penicillin and streptomycin. The MSV vector containing the gene is then added to the media and the packaging cells transduced with the vector.  
20 The packaging cells now produce infectious viral particles containing the gene (the packaging cells are now referred to as producer cells).

          Fresh media is added to the transduced producer cells, and subsequently, the media is harvested from a 10 cm plate of confluent producer cells. The spent media, containing the infectious viral particles, is filtered through a millipore filter to remove  
25 detached producer cells and this media is then used to infect fibroblast cells. Media is removed from a sub-confluent plate of fibroblasts and quickly replaced with the

media from the producer cells. This media is removed and replaced with fresh media. If the titer of virus is high, then virtually all fibroblasts will be infected and no selection is required. If the titer is very low, then it is necessary to use a retroviral vector that has a selectable marker, such as neo or his. Once the fibroblasts have been efficiently infected, the fibroblasts are analyzed to determine whether protein is produced.

The engineered fibroblasts are then transplanted onto the host, either alone or after having been grown to confluence on cytodex 3 microcarrier beads.

10 **Example 27: Gene Therapy Using Endogenous Genes Corresponding To Polynucleotides of the Invention**

Another method of gene therapy according to the present invention involves operably associating the endogenous polynucleotide sequence of the invention with a promoter via homologous recombination as described, for example, in U.S. Patent NO: 5,641,670, issued June 24, 1997; International Publication NO: WO 96/29411, published September 26, 1996; International Publication NO: WO 94/12650, published August 4, 1994; Koller et al., *Proc. Natl. Acad. Sci. USA*, 86:8932-8935 (1989); and Zijlstra et al., *Nature*, 342:435-438 (1989). This method involves the activation of a gene which is present in the target cells, but which is not expressed in the cells, or is expressed at a lower level than desired.

Polynucleotide constructs are made which contain a promoter and targeting sequences, which are homologous to the 5' non-coding sequence of endogenous polynucleotide sequence, flanking the promoter. The targeting sequence will be sufficiently near the 5' end of the polynucleotide sequence so the promoter will be operably linked to the endogenous sequence upon homologous recombination. The promoter and the targeting sequences can be amplified using PCR. Preferably, the

amplified promoter contains distinct restriction enzyme sites on the 5' and 3' ends. Preferably, the 3' end of the first targeting sequence contains the same restriction enzyme site as the 5' end of the amplified promoter and the 5' end of the second targeting sequence contains the same restriction site as the 3' end of the amplified promoter.

The amplified promoter and the amplified targeting sequences are digested with the appropriate restriction enzymes and subsequently treated with calf intestinal phosphatase. The digested promoter and digested targeting sequences are added together in the presence of T4 DNA ligase. The resulting mixture is maintained under conditions appropriate for ligation of the two fragments. The construct is size fractionated on an agarose gel then purified by phenol extraction and ethanol precipitation.

In this Example, the polynucleotide constructs are administered as naked polynucleotides via electroporation. However, the polynucleotide constructs may also be administered with transfection-facilitating agents, such as liposomes, viral sequences, viral particles, precipitating agents, etc. Such methods of delivery are known in the art.

Once the cells are transfected, homologous recombination will take place which results in the promoter being operably linked to the endogenous polynucleotide sequence. This results in the expression of polynucleotide corresponding to the polynucleotide in the cell. Expression may be detected by immunological staining, or any other method known in the art.

Fibroblasts are obtained from a subject by skin biopsy. The resulting tissue is placed in DMEM + 10% fetal calf serum. Exponentially growing or early stationary phase fibroblasts are trypsinized and rinsed from the plastic surface with nutrient medium. An aliquot of the cell suspension is removed for counting, and the remaining

cells are subjected to centrifugation. The supernatant is aspirated and the pellet is resuspended in 5 ml of electroporation buffer (20 mM HEPES pH 7.3, 137 mM NaCl, 5 mM KCl, 0.7 mM Na<sub>2</sub> HPO<sub>4</sub>, 6 mM dextrose). The cells are recentrifuged, the supernatant aspirated, and the cells resuspended in electroporation buffer containing 1 mg/ml acetylated bovine serum albumin. The final cell suspension contains approximately 3X10<sup>6</sup> cells/ml. Electroporation should be performed immediately following resuspension.

Plasmid DNA is prepared according to standard techniques. For example, to construct a plasmid for targeting to the locus corresponding to the polynucleotide of the invention, plasmid pUC18 (MBI Fermentas, Amherst, NY) is digested with HindIII. The CMV promoter is amplified by PCR with an XbaI site on the 5' end and a BamHI site on the 3'end. Two non-coding sequences are amplified via PCR: one non-coding sequence (fragment 1) is amplified with a HindIII site at the 5' end and an Xba site at the 3'end; the other non-coding sequence (fragment 2) is amplified with a BamHI site at the 5'end and a HindIII site at the 3'end. The CMV promoter and the fragments (1 and 2) are digested with the appropriate enzymes (CMV promoter - XbaI and BamHI; fragment 1 - XbaI; fragment 2 - BamHI) and ligated together. The resulting ligation product is digested with HindIII, and ligated with the HindIII-digested pUC18 plasmid.

Plasmid DNA is added to a sterile cuvette with a 0.4 cm electrode gap (Bio-Rad). The final DNA concentration is generally at least 120 µg/ml. 0.5 ml of the cell suspension (containing approximately 1.5X10<sup>6</sup> cells) is then added to the cuvette, and the cell suspension and DNA solutions are gently mixed. Electroporation is performed with a Gene-Pulser apparatus (Bio-Rad). Capacitance and voltage are set at 960 µF and 250-300 V, respectively. As voltage increases, cell survival decreases, but the percentage of surviving cells that stably incorporate the introduced DNA into their



genome increases dramatically. Given these parameters, a pulse time of approximately 14-20 mSec should be observed.

Electroporated cells are maintained at room temperature for approximately 5 min, and the contents of the cuvette are then gently removed with a sterile transfer  
5 pipette. The cells are added directly to 10 ml of prewarmed nutrient media (DMEM with 15% calf serum) in a 10 cm dish and incubated at 37 degree C. The following day, the media is aspirated and replaced with 10 ml of fresh media and incubated for a further 16-24 hours.

The engineered fibroblasts are then injected into the host, either alone or after  
10 having been grown to confluence on cytodex 3 microcarrier beads. The fibroblasts now produce the protein product. The fibroblasts can then be introduced into a patient as described above.

**Example 28: Method of Treatment Using Gene Therapy - In Vivo**

15 Another aspect of the present invention is using *in vivo* gene therapy methods to treat disorders, diseases and conditions. The gene therapy method relates to the introduction of naked nucleic acid (DNA, RNA, and antisense DNA or RNA) sequences into an animal to increase or decrease the expression of the polypeptide. The polynucleotide of the present invention may be operatively linked to a promoter  
20 or any other genetic elements necessary for the expression of the polypeptide by the target tissue. Such gene therapy and delivery techniques and methods are known in the art, see, for example, WO90/11092, WO98/11779; U.S. Patent NO. 5693622, 5705151, 5580859; Tabata et al., Cardiovasc. Res. 35(3):470-479 (1997); Chao et al., Pharmacol. Res. 35(6):517-522 (1997); Wolff, Neuromuscul. Disord. 7(5):314-318  
25 (1997); Schwartz et al., Gene Ther. 3(5):405-411 (1996); Tsurumi et al., Circulation 94(12):3281-3290 (1996) (incorporated herein by reference).

The polynucleotide constructs may be delivered by any method that delivers injectable materials to the cells of an animal, such as, injection into the interstitial space of tissues (heart, muscle, skin, lung, liver, intestine and the like). The polynucleotide constructs can be delivered in a pharmaceutically acceptable liquid or aqueous carrier.

The term "naked" polynucleotide, DNA or RNA, refers to sequences that are free from any delivery vehicle that acts to assist, promote, or facilitate entry into the cell, including viral sequences, viral particles, liposome formulations, lipofectin or precipitating agents and the like. However, the polynucleotides of the present invention may also be delivered in liposome formulations (such as those taught in Felgner P.L. et al. (1995) Ann. NY Acad. Sci. 772:126-139 and Abdallah B. et al. (1995) Biol. Cell 85(1):1-7) which can be prepared by methods well known to those skilled in the art.

The polynucleotide vector constructs used in the gene therapy method are preferably constructs that will not integrate into the host genome nor will they contain sequences that allow for replication. Any strong promoter known to those skilled in the art can be used for driving the expression of DNA. Unlike other gene therapies techniques, one major advantage of introducing naked nucleic acid sequences into target cells is the transitory nature of the polynucleotide synthesis in the cells. Studies have shown that non-replicating DNA sequences can be introduced into cells to provide production of the desired polypeptide for periods of up to six months.

The polynucleotide construct can be delivered to the interstitial space of tissues within the an animal, including of muscle, skin, brain, lung, liver, spleen, bone marrow, thymus, heart, lymph, blood, bone, cartilage, pancreas, kidney, gall bladder, stomach, intestine, testis, ovary, uterus, rectum, nervous system, eye, gland, and connective tissue. Interstitial space of the tissues comprises the intercellular fluid,

mucopolysaccharide matrix among the reticular fibers of organ tissues, elastic fibers in the walls of vessels or chambers, collagen fibers of fibrous tissues, or that same matrix within connective tissue ensheathing muscle cells or in the lacunae of bone. It is similarly the space occupied by the plasma of the circulation and the lymph fluid of the lymphatic channels. Delivery to the interstitial space of muscle tissue is preferred for the reasons discussed below. They may be conveniently delivered by injection into the tissues comprising these cells. They are preferably delivered to and expressed in persistent, non-dividing cells which are differentiated, although delivery and expression may be achieved in non-differentiated or less completely differentiated cells, such as, for example, stem cells of blood or skin fibroblasts. *In vivo* muscle cells are particularly competent in their ability to take up and express polynucleotides.

For the naked polynucleotide injection, an effective dosage amount of DNA or RNA will be in the range of from about 0.05 g/kg body weight to about 50 mg/kg body weight. Preferably the dosage will be from about 0.005 mg/kg to about 20 mg/kg and more preferably from about 0.05 mg/kg to about 5 mg/kg. Of course, as the artisan of ordinary skill will appreciate, this dosage will vary according to the tissue site of injection. The appropriate and effective dosage of nucleic acid sequence can readily be determined by those of ordinary skill in the art and may depend on the condition being treated and the route of administration. The preferred route of administration is by the parenteral route of injection into the interstitial space of tissues. However, other parenteral routes may also be used, such as, inhalation of an aerosol formulation particularly for delivery to lungs or bronchial tissues, throat or mucous membranes of the nose. In addition, naked polynucleotide constructs can be delivered to arteries during angioplasty by the catheter used in the procedure.

The dose response effects of injected polynucleotide in muscle *in vivo* is

determined as follows. Suitable template DNA for production of mRNA coding for polypeptide of the present invention is prepared in accordance with a standard recombinant DNA methodology. The template DNA, which may be either circular or linear, is either used as naked DNA or complexed with liposomes. The quadriceps  
5 muscles of mice are then injected with various amounts of the template DNA.

Five to six week old female and male Balb/C mice are anesthetized by intraperitoneal injection with 0.3 ml of 2.5% Avertin. A 1.5 cm incision is made on the anterior thigh, and the quadriceps muscle is directly visualized. The template DNA is injected in 0.1 ml of carrier in a 1 cc syringe through a 27 gauge needle over  
10 one minute, approximately 0.5 cm from the distal insertion site of the muscle into the knee and about 0.2 cm deep. A suture is placed over the injection site for future localization, and the skin is closed with stainless steel clips.

After an appropriate incubation time (e.g., 7 days) muscle extracts are prepared by excising the entire quadriceps. Every fifth 15 um cross-section of the  
15 individual quadriceps muscles is histochemically stained for protein expression. A time course for protein expression may be done in a similar fashion except that quadriceps from different mice are harvested at different times. Persistence of DNA in muscle following injection may be determined by Southern blot analysis after preparing total cellular DNA and HIRT supernatants from injected and control mice.  
20 The results of the above experimentation in mice can be use to extrapolate proper dosages and other treatment parameters in humans and other animals using naked DNA.

#### **Example 29: Transgenic Animals.**

25 The polypeptides of the invention can also be expressed in transgenic animals. Animals of any species, including, but not limited to, mice, rats, rabbits, hamsters,

guinea pigs, pigs, micro-pigs, goats, sheep, cows and non-human primates, *e.g.*, baboons, monkeys, and chimpanzees may be used to generate transgenic animals. In a specific embodiment, techniques described herein or otherwise known in the art, are used to express polypeptides of the invention in humans, as part of a gene therapy  
5 protocol.

Any technique known in the art may be used to introduce the transgene (*i.e.*, polynucleotides of the invention) into animals to produce the founder lines of transgenic animals. Such techniques include, but are not limited to, pronuclear microinjection (Paterson et al., Appl. Microbiol. Biotechnol. 40:691-698 (1994);  
10 Carver et al., Biotechnology (NY) 11:1263-1270 (1993); Wright et al., Biotechnology (NY) 9:830-834 (1991); and Hoppe et al., U.S. Pat. No. 4,873,191 (1989)); retrovirus mediated gene transfer into germ lines (Van der Putten et al., Proc. Natl. Acad. Sci., USA 82:6148-6152 (1985)), blastocysts or embryos; gene targeting in embryonic stem cells (Thompson et al., Cell 56:313-321 (1989)); electroporation of cells or  
15 embryos (Lo, 1983, Mol Cell. Biol. 3:1803-1814 (1983)); introduction of the polynucleotides of the invention using a gene gun (see, *e.g.*, Ulmer et al., Science 259:1745 (1993)); introducing nucleic acid constructs into embryonic pluripotent stem cells and transferring the stem cells back into the blastocyst; and sperm-mediated gene transfer (Lavitrano et al., Cell 57:717-723 (1989)); etc. For a review of  
20 such techniques, see Gordon, "Transgenic Animals," Intl. Rev. Cytol. 115:171-229 (1989), which is incorporated by reference herein in its entirety.

Any technique known in the art may be used to produce transgenic clones containing polynucleotides of the invention, for example, nuclear transfer into enucleated oocytes of nuclei from cultured embryonic, fetal, or adult cells induced to  
25 quiescence (Campell et al., Nature 380:64-66 (1996); Wilmut et al., Nature 385:810-813 (1997)).

The present invention provides for transgenic animals that carry the transgene in all their cells, as well as animals which carry the transgene in some, but not all their cells, *i.e.*, mosaic animals or chimeric. The transgene may be integrated as a single transgene or as multiple copies such as in concatamers, *e.g.*, head-to-head tandems or head-to-tail tandems. The transgene may also be selectively introduced into and activated in a particular cell type by following, for example, the teaching of Lasko et al. (Lasko et al., Proc. Natl. Acad. Sci. USA 89:6232-6236 (1992)). The regulatory sequences required for such a cell-type specific activation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art. When it is desired that the polynucleotide transgene be integrated into the chromosomal site of the endogenous gene, gene targeting is preferred. Briefly, when such a technique is to be utilized, vectors containing some nucleotide sequences homologous to the endogenous gene are designed for the purpose of integrating, via homologous recombination with chromosomal sequences, into and disrupting the function of the nucleotide sequence of the endogenous gene. The transgene may also be selectively introduced into a particular cell type, thus inactivating the endogenous gene in only that cell type, by following, for example, the teaching of Gu et al. (Gu et al., Science 265:103-106 (1994)). The regulatory sequences required for such a cell-type specific inactivation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art.

Once transgenic animals have been generated, the expression of the recombinant gene may be assayed utilizing standard techniques. Initial screening may be accomplished by Southern blot analysis or PCR techniques to analyze animal tissues to verify that integration of the transgene has taken place. The level of mRNA expression of the transgene in the tissues of the transgenic animals may also be assessed using techniques which include, but are not limited to, Northern blot analysis

of tissue samples obtained from the animal, *in situ* hybridization analysis, and reverse transcriptase-PCR (rt-PCR). Samples of transgenic gene-expressing tissue may also be evaluated immunocytochemically or immunohistochemically using antibodies specific for the transgene product.

5           Once the founder animals are produced, they may be bred, inbred, outbred, or crossbred to produce colonies of the particular animal. Examples of such breeding strategies include, but are not limited to: outbreeding of founder animals with more than one integration site in order to establish separate lines; inbreeding of separate lines in order to produce compound transgenics that express the transgene at higher  
10 levels because of the effects of additive expression of each transgene; crossing of heterozygous transgenic animals to produce animals homozygous for a given integration site in order to both augment expression and eliminate the need for screening of animals by DNA analysis; crossing of separate homozygous lines to produce compound heterozygous or homozygous lines; and breeding to place the  
15 transgene on a distinct background that is appropriate for an experimental model of interest.

Transgenic animals of the invention have uses which include, but are not limited to, animal model systems useful in elaborating the biological function of polypeptides of the present invention, studying diseases, disorders, and/or conditions  
20 associated with aberrant expression, and in screening for compounds effective in ameliorating such diseases, disorders, and/or conditions.

#### **Example 30: Knock-Out Animals.**

Endogenous gene expression can also be reduced by inactivating or "knocking  
25 out" the gene and/or its promoter using targeted homologous recombination. (*E.g.*, see Smithies et al., *Nature* 317:230-234 (1985); Thomas & Capecchi, *Cell* 51:503-

512 (1987); Thompson et al., Cell 5:313-321 (1989); each of which is incorporated by reference herein in its entirety). For example, a mutant, non-functional polynucleotide of the invention (or a completely unrelated DNA sequence) flanked by DNA homologous to the endogenous polynucleotide sequence (either the coding regions or regulatory regions of the gene) can be used, with or without a selectable marker and/or a negative selectable marker, to transfect cells that express polypeptides of the invention *in vivo*. In another embodiment, techniques known in the art are used to generate knockouts in cells that contain, but do not express the gene of interest. Insertion of the DNA construct, via targeted homologous recombination, results in inactivation of the targeted gene. Such approaches are particularly suited in research and agricultural fields where modifications to embryonic stem cells can be used to generate animal offspring with an inactive targeted gene (*e.g.*, see Thomas & Capecchi 1987 and Thompson 1989, *supra*). However this approach can be routinely adapted for use in humans provided the recombinant DNA constructs are directly administered or targeted to the required site *in vivo* using appropriate viral vectors that will be apparent to those of skill in the art.

In further embodiments of the invention, cells that are genetically engineered to express the polypeptides of the invention, or alternatively, that are genetically engineered not to express the polypeptides of the invention (*e.g.*, knockouts) are administered to a patient *in vivo*. Such cells may be obtained from the patient (*i.e.*, animal, including human) or an MHC compatible donor and can include, but are not limited to fibroblasts, bone marrow cells, blood cells (*e.g.*, lymphocytes), adipocytes, muscle cells, endothelial cells etc. The cells are genetically engineered *in vitro* using recombinant DNA techniques to introduce the coding sequence of polypeptides of the invention into the cells, or alternatively, to disrupt the coding sequence and/or endogenous regulatory sequence associated with the polypeptides of the invention,



e.g., by transduction (using viral vectors, and preferably vectors that integrate the transgene into the cell genome) or transfection procedures, including, but not limited to, the use of plasmids, cosmids, YACs, naked DNA, electroporation, liposomes, etc. The coding sequence of the polypeptides of the invention can be placed under the control of a strong constitutive or inducible promoter or promoter/enhancer to achieve expression, and preferably secretion, of the polypeptides of the invention. The engineered cells which express and preferably secrete the polypeptides of the invention can be introduced into the patient systemically, e.g., in the circulation, or intraperitoneally.

Alternatively, the cells can be incorporated into a matrix and implanted in the body, e.g., genetically engineered fibroblasts can be implanted as part of a skin graft; genetically engineered endothelial cells can be implanted as part of a lymphatic or vascular graft. (See, for example, Anderson et al. U.S. Patent No. 5,399,349; and Mulligan & Wilson, U.S. Patent No. 5,460,959 each of which is incorporated by reference herein in its entirety).

When the cells to be administered are non-autologous or non-MHC compatible cells, they can be administered using well known techniques which prevent the development of a host immune response against the introduced cells. For example, the cells may be introduced in an encapsulated form which, while allowing for an exchange of components with the immediate extracellular environment, does not allow the introduced cells to be recognized by the host immune system.

Transgenic and "knock-out" animals of the invention have uses which include, but are not limited to, animal model systems useful in elaborating the biological function of polypeptides of the present invention, studying diseases, disorders, and/or conditions associated with aberrant expression, and in screening for compounds effective in ameliorating such diseases, disorders, and/or conditions.

**Example 31: Production of an Antibody****a) Hybridoma Technology**

The antibodies of the present invention can be prepared by a variety of methods. (See, Current Protocols, Chapter 2.) As one example of such methods, cells expressing XXX are administered to an animal to induce the production of sera containing polyclonal antibodies. In a preferred method, a preparation of XXX protein is prepared and purified to render it substantially free of natural contaminants. Such a preparation is then introduced into an animal in order to produce polyclonal antisera of greater specific activity.

Monoclonal antibodies specific for protein XXX are prepared using hybridoma technology. (Kohler et al., Nature 256:495 (1975); Kohler et al., Eur. J. Immunol. 6:511 (1976); Kohler et al., Eur. J. Immunol. 6:292 (1976); Hammerling et al., in: Monoclonal Antibodies and T-Cell Hybridomas, Elsevier, N.Y., pp. 563-681 (1981)). In general, an animal (preferably a mouse) is immunized with XXX polypeptide or, more preferably, with a secreted XXX polypeptide-expressing cell. Such polypeptide-expressing cells are cultured in any suitable tissue culture medium, preferably in Earle's modified Eagle's medium supplemented with 10% fetal bovine serum (inactivated at about 56°C), and supplemented with about 10 g/l of nonessential amino acids, about 1,000 U/ml of penicillin, and about 100 µg/ml of streptomycin.

The splenocytes of such mice are extracted and fused with a suitable myeloma cell line. Any suitable myeloma cell line may be employed in accordance with the present invention; however, it is preferable to employ the parent myeloma cell line (SP2O), available from the ATCC. After fusion, the resulting hybridoma cells are selectively maintained in HAT medium, and then cloned by limiting dilution as

described by Wands et al. (Gastroenterology 80:225-232 (1981)). The hybridoma cells obtained through such a selection are then assayed to identify clones which secrete antibodies capable of binding the XXX polypeptide.

Alternatively, additional antibodies capable of binding to XXX polypeptide  
5 can be produced in a two-step procedure using anti-idiotypic antibodies. Such a method makes use of the fact that antibodies are themselves antigens, and therefore, it is possible to obtain an antibody which binds to a second antibody. In accordance with this method, protein specific antibodies are used to immunize an animal, preferably a mouse. The splenocytes of such an animal are then used to produce  
10 hybridoma cells, and the hybridoma cells are screened to identify clones which produce an antibody whose ability to bind to the XXX protein-specific antibody can be blocked by XXX. Such antibodies comprise anti-idiotypic antibodies to the XXX protein-specific antibody and are used to immunize an animal to induce formation of further XXX protein-specific antibodies.

15 For in vivo use of antibodies in humans, an antibody is "humanized". Such antibodies can be produced using genetic constructs derived from hybridoma cells producing the monoclonal antibodies described above. Methods for producing chimeric and humanized antibodies are known in the art and are discussed herein. (See, for review, Morrison, Science 229:1202 (1985); Oi et al., BioTechniques 4:214  
20 (1986); Cabilly et al., U.S. Patent No. 4,816,567; Taniguchi et al., EP 171496; Morrison et al., EP 173494; Neuberger et al., WO 8601533; Robinson et al., WO 8702671; Boulianne et al., Nature 312:643 (1984); Neuberger et al., Nature 314:268 (1985).)

25           b) Isolation Of Antibody Fragments Directed  
              Against XXX From A Library Of scFvs

Naturally occurring V-genes isolated from human PBLs are constructed into a library of antibody fragments which contain reactivities against XXX to which the donor may or may not have been exposed (see e.g., U.S. Patent 5,885,793 incorporated herein by reference in its entirety).

- 5           Rescue of the Library. A library of scFvs is constructed from the RNA of human PBLs as described in PCT publication WO 92/01047. To rescue phage displaying antibody fragments, approximately 10<sup>9</sup> E. coli harboring the phagemid are used to inoculate 50 ml of 2xTY containing 1% glucose and 100 µg/ml of ampicillin (2xTY-AMP-GLU) and grown to an O.D. of 0.8 with shaking. Five ml of this culture  
10 is used to inoculate 50 ml of 2xTY-AMP-GLU, 2 x 10<sup>8</sup> TU of delta gene 3 helper (M13 delta gene III, see PCT publication WO 92/01047) are added and the culture incubated at 37°C for 45 minutes without shaking and then at 37°C for 45 minutes with shaking. The culture is centrifuged at 4000 r.p.m. for 10 min. and the pellet resuspended in 2 liters of 2xTY containing 100 µg/ml ampicillin and 50 µg/ml  
15 kanamycin and grown overnight. Phage are prepared as described in PCT publication WO 92/01047.

- M13 delta gene III is prepared as follows: M13 delta gene III helper phage does not encode gene III protein, hence the phage(mid) displaying antibody fragments have a greater avidity of binding to antigen. Infectious M13 delta gene III  
20 particles are made by growing the helper phage in cells harboring a pUC19 derivative supplying the wild type gene III protein during phage morphogenesis. The culture is incubated for 1 hour at 37° C without shaking and then for a further hour at 37°C with shaking. Cells are spun down (IEC-Centra 8,400 r.p.m. for 10 min), resuspended in 300 ml 2xTY broth containing 100 µg ampicillin/ml and 25 µg kanamycin/ml (2xTY-  
25 AMP-KAN) and grown overnight, shaking at 37°C. Phage particles are purified and concentrated from the culture medium by two PEG-precipitations (Sambrook et al.,

1990), resuspended in 2 ml PBS and passed through a 0.45  $\mu$ m filter (Minisart NML; Sartorius) to give a final concentration of approximately  $10^{13}$  transducing units/ml (ampicillin-resistant clones).

Panning of the Library. Immunotubes (Nunc) are coated overnight in PBS with 4 ml of either 100  $\mu$ g/ml or 10  $\mu$ g/ml of a polypeptide of the present invention. Tubes are blocked with 2% Marvel-PBS for 2 hours at 37°C and then washed 3 times in PBS. Approximately  $10^{13}$  TU of phage is applied to the tube and incubated for 30 minutes at room temperature tumbling on an over and under turntable and then left to stand for another 1.5 hours. Tubes are washed 10 times with PBS 0.1% Tween-20 and 10 times with PBS. Phage are eluted by adding 1 ml of 100 mM triethylamine and rotating 15 minutes on an under and over turntable after which the solution is immediately neutralized with 0.5 ml of 1.0M Tris-HCl, pH 7.4. Phage are then used to infect 10 ml of mid-log E. coli TG1 by incubating eluted phage with bacteria for 30 minutes at 37°C. The E. coli are then plated on TYE plates containing 1% glucose and 100  $\mu$ g/ml ampicillin. The resulting bacterial library is then rescued with delta gene 3 helper phage as described above to prepare phage for a subsequent round of selection. This process is then repeated for a total of 4 rounds of affinity purification with tube-washing increased to 20 times with PBS, 0.1% Tween-20 and 20 times with PBS for rounds 3 and 4.

Characterization of Binders. Eluted phage from the 3rd and 4th rounds of selection are used to infect E. coli HB 2151 and soluble scFv is produced (Marks, et al., 1991) from single colonies for assay. ELISAs are performed with microtitre plates coated with either 10 pg/ml of the polypeptide of the present invention in 50 mM bicarbonate pH 9.6. Clones positive in ELISA are further characterized by PCR fingerprinting (see, e.g., PCT publication WO 92/01047) and then by sequencing. These ELISA positive clones may also be further characterized by techniques known

in the art, such as, for example, epitope mapping, binding affinity, receptor signal transduction, ability to block or competitively inhibit antibody/antigen binding, and competitive agonistic or antagonistic activity.

5    **Example 32: Assays Detecting Stimulation or Inhibition of B cell Proliferation and Differentiation**

Generation of functional humoral immune responses requires both soluble and cognate signaling between B-lineage cells and their microenvironment. Signals may  
10    impart a positive stimulus that allows a B-lineage cell to continue its programmed development, or a negative stimulus that instructs the cell to arrest its current developmental pathway. To date, numerous stimulatory and inhibitory signals have been found to influence B cell responsiveness including IL-2, IL-4, IL-5, IL-6, IL-7, IL10, IL-13, IL-14 and IL-15. Interestingly, these signals are by themselves weak effectors but can,  
15    in combination with various co-stimulatory proteins, induce activation, proliferation, differentiation, homing; tolerance and death among B cell populations.

One of the best studied classes of B-cell co-stimulatory proteins is the TNF-superfamily. Within this family CD40, CD27, and CD30 along with their respective ligands CD154, CD70, and CD153 have been found to regulate a variety of immune  
20    responses. Assays which allow for the detection and/or observation of the proliferation and differentiation of these B-cell populations and their precursors are valuable tools in determining the effects various proteins may have on these B-cell populations in terms of proliferation and differentiation. Listed below are two assays designed to allow for the detection of the differentiation, proliferation, or inhibition of B-cell populations and their  
25    precursors.

In Vitro Assay- Purified polypeptides of the invention, or truncated forms thereof, is assessed for its ability to induce activation, proliferation, differentiation or

inhibition and/or death in B-cell populations and their precursors. The activity of the polypeptides of the invention on purified human tonsillar B cells, measured qualitatively over the dose range from 0.1 to 10,000 ng/mL, is assessed in a standard B-lymphocyte co-stimulation assay in which purified tonsillar B cells are cultured in the presence of either formalin-fixed *Staphylococcus aureus* Cowan I (SAC) or immobilized anti-human IgM antibody as the priming agent. Second signals such as IL-2 and IL-15 synergize with SAC and IgM crosslinking to elicit B cell proliferation as measured by tritiated-thymidine incorporation. Novel synergizing agents can be readily identified using this assay. The assay involves isolating human tonsillar B cells by magnetic bead (MACS) depletion of CD3-positive cells. The resulting cell population is greater than 95% B cells as assessed by expression of CD45R(B220).

Various dilutions of each sample are placed into individual wells of a 96-well plate to which are added  $10^5$  B-cells suspended in culture medium (RPMI 1640 containing 10% FBS,  $5 \times 10^{-5}$ M 2ME, 100U/ml penicillin, 10ug/ml streptomycin, and  $10^{-5}$  dilution of SAC) in a total volume of 150ul. Proliferation or inhibition is quantitated by a 20h pulse (1uCi/well) with 3H-thymidine (6.7 Ci/mM) beginning 72h post factor addition. The positive and negative controls are IL2 and medium respectively.

In Vivo Assay- BALB/c mice are injected (i.p.) twice per day with buffer only, or 2 mg/Kg of a polypeptide of the invention, or truncated forms thereof. Mice receive this treatment for 4 consecutive days, at which time they are sacrificed and various tissues and serum collected for analyses. Comparison of H&E sections from normal spleens and spleens treated with polypeptides of the invention identify the results of the activity of the polypeptides on spleen cells, such as the diffusion of per-arterial lymphatic sheaths, and/or significant increases in the nucleated cellularity of the red pulp regions, which may indicate the activation of the differentiation and proliferation of B-cell populations. Immunohistochemical studies using a B cell

marker, anti-CD45R(B220), are used to determine whether any physiological changes to splenic cells, such as splenic disorganization, are due to increased B-cell representation within loosely defined B-cell zones that infiltrate established T-cell regions.

5           Flow cytometric analyses of the spleens from mice treated with polypeptide is used to indicate whether the polypeptide specifically increases the proportion of ThB+, CD45R(B220)dull B cells over that which is observed in control mice.

          Likewise, a predicted consequence of increased mature B-cell representation in vivo is a relative increase in serum Ig titers. Accordingly, serum IgM and IgA levels are  
10       compared between buffer and polypeptide-treated mice.

          The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides of the invention (e.g., gene therapy), agonists, and/or antagonists of polynucleotides or polypeptides of the invention.

15

### **Example 33: T Cell Proliferation Assay**

          A CD3-induced proliferation assay is performed on PBMCs and is measured by the uptake of <sup>3</sup>H-thymidine. The assay is performed as follows. Ninety-six well plates are coated with 100 µl/well of mAb to CD3 (HIT3a, Pharmingen) or isotype-matched control  
20       mAb (B33.1) overnight at 4 degrees C (1 µg/ml in .05M bicarbonate buffer, pH 9.5), then washed three times with PBS. PBMC are isolated by F/H gradient centrifugation from human peripheral blood and added to quadruplicate wells (5 x 10<sup>4</sup>/well) of mAb coated plates in RPMI containing 10% FCS and P/S in the presence of varying concentrations of polypeptides of the invention (total volume 200 ul). Relevant protein buffer and medium  
25       alone are controls. After 48 hr. culture at 37 degrees C, plates are spun for 2 min. at 1000 rpm and 100 µl of supernatant is removed and stored -20 degrees C for measurement of



IL-2 (or other cytokines) if effect on proliferation is observed. Wells are supplemented with 100 ul of medium containing 0.5 uCi of  $^3\text{H}$ -thymidine and cultured at 37 degrees C for 18-24 hr. Wells are harvested and incorporation of  $^3\text{H}$ -thymidine used as a measure of proliferation. Anti-CD3 alone is the positive control for proliferation. IL-2 (100 U/ml) is  
5 also used as a control which enhances proliferation. Control antibody which does not induce proliferation of T cells is used as the negative controls for the effects of polypeptides of the invention.

The studies described in this example tested activity of polypeptides of the invention. However, one skilled in the art could easily modify the exemplified studies to  
10 test the activity of polynucleotides of the invention (e.g., gene therapy), agonists, and/or antagonists of polynucleotides or polypeptides of the invention.

**Example 34: Effect of Polypeptides of the Invention on the Expression of MHC Class II, Costimulatory and Adhesion Molecules and Cell Differentiation of**  
15 **Monocytes and Monocyte-Derived Human Dendritic Cells**

Dendritic cells are generated by the expansion of proliferating precursors found in the peripheral blood: adherent PBMC or elutriated monocytic fractions are cultured for 7-10 days with GM-CSF (50 ng/ml) and IL-4 (20 ng/ml). These dendritic cells have the  
20 characteristic phenotype of immature cells (expression of CD1, CD80, CD86, CD40 and MHC class II antigens). Treatment with activating factors, such as  $\text{TNF-}\alpha$ , causes a rapid change in surface phenotype (increased expression of MHC class I and II, costimulatory and adhesion molecules, downregulation of  $\text{FC}\gamma\text{RII}$ , upregulation of CD83). These changes correlate with increased antigen-presenting capacity and with functional  
25 maturation of the dendritic cells.

FACS analysis of surface antigens is performed as follows. Cells are treated 1-3 days with increasing concentrations of polypeptides of the invention or LPS (positive

control), washed with PBS containing 1% BSA and 0.02 mM sodium azide, and then incubated with 1:20 dilution of appropriate FITC- or PE-labeled monoclonal antibodies for 30 minutes at 4 degrees C. After an additional wash, the labeled cells are analyzed by flow cytometry on a FACScan (Becton Dickinson).

5

Effect on the production of cytokines. Cytokines generated by dendritic cells, in particular IL-12, are important in the initiation of T-cell dependent immune responses. IL-12 strongly influences the development of Th1 helper T-cell immune response, and induces cytotoxic T and NK cell function. An ELISA is used to measure the IL-12 release as follows. Dendritic cells ( $10^6$ /ml) are treated with increasing concentrations of polypeptides of the invention for 24 hours. LPS (100 ng/ml) is added to the cell culture as positive control. Supernatants from the cell cultures are then collected and analyzed for IL-12 content using commercial ELISA kit (e.g., R & D Systems (Minneapolis, MN)). The standard protocols provided with the kits are used.

15

Effect on the expression of MHC Class II, costimulatory and adhesion molecules. Three major families of cell surface antigens can be identified on monocytes: adhesion molecules, molecules involved in antigen presentation, and Fc receptor. Modulation of the expression of MHC class II antigens and other costimulatory molecules, such as B7 and ICAM-1, may result in changes in the antigen presenting capacity of monocytes and ability to induce T cell activation. Increase expression of Fc receptors may correlate with improved monocyte cytotoxic activity, cytokine release and phagocytosis.

20

FACS analysis is used to examine the surface antigens as follows. Monocytes are treated 1-5 days with increasing concentrations of polypeptides of the invention or

25

LPS (positive control), washed with PBS containing 1% BSA and 0.02 mM sodium azide, and then incubated with 1:20 dilution of appropriate FITC- or PE-labeled monoclonal antibodies for 30 minutes at 4 degreesC. After an additional wash, the labeled cells are analyzed by flow cytometry on a FACScan (Becton Dickinson).

5

Monocyte activation and/or increased survival. Assays for molecules that activate (or alternatively, inactivate) monocytes and/or increase monocyte survival (or alternatively, decrease monocyte survival) are known in the art and may routinely be applied to determine whether a molecule of the invention functions as an inhibitor or  
10 activator of monocytes. Polypeptides, agonists, or antagonists of the invention can be screened using the three assays described below. For each of these assays, Peripheral blood mononuclear cells (PBMC) are purified from single donor leukopacks (American Red Cross, Baltimore, MD) by centrifugation through a Histopaque gradient (Sigma). Monocytes are isolated from PBMC by counterflow centrifugal  
15 elutriation.

Monocyte Survival Assay. Human peripheral blood monocytes progressively lose viability when cultured in absence of serum or other stimuli. Their death results from internally regulated process (apoptosis). Addition to the culture of activating  
20 factors, such as TNF-alpha dramatically improves cell survival and prevents DNA fragmentation. Propidium iodide (PI) staining is used to measure apoptosis as follows. Monocytes are cultured for 48 hours in polypropylene tubes in serum-free medium (positive control), in the presence of 100 ng/ml TNF-alpha (negative control), and in the presence of varying concentrations of the compound to be tested.  
25 Cells are suspended at a concentration of  $2 \times 10^6$ /ml in PBS containing PI at a final concentration of 5  $\mu$ g/ml, and then incubated at room temperature for 5 minutes before

FACScan analysis. PI uptake has been demonstrated to correlate with DNA fragmentation in this experimental paradigm.

Effect on cytokine release. An important function of monocytes/macrophages is their regulatory activity on other cellular populations of the immune system through the release of cytokines after stimulation. An ELISA to measure cytokine release is performed as follows. Human monocytes are incubated at a density of  $5 \times 10^5$  cells/ml with increasing concentrations of the a polypeptide of the invention and under the same conditions, but in the absence of the polypeptide. For IL-12 production, the cells are primed overnight with IFN (100 U/ml) in presence of a polypeptide of the invention. LPS (10 ng/ml) is then added. Conditioned media are collected after 24h and kept frozen until use. Measurement of TNF-alpha, IL-10, MCP-1 and IL-8 is then performed using a commercially available ELISA kit (e.g., R & D Systems (Minneapolis, MN)) and applying the standard protocols provided with the kit.

15

Oxidative burst. Purified monocytes are plated in 96-w plate at  $2 \times 10^5$  cell/well. Increasing concentrations of polypeptides of the invention are added to the wells in a total volume of 0.2 ml culture medium (RPMI 1640 + 10% FCS, glutamine and antibiotics). After 3 days incubation, the plates are centrifuged and the medium is removed from the wells. To the macrophage monolayers, 0.2 ml per well of phenol red solution (140 mM NaCl, 10 mM potassium phosphate buffer pH 7.0, 5.5 mM dextrose, 0.56 mM phenol red and 19 U/ml of HRPO) is added, together with the stimulant (200 nM PMA). The plates are incubated at 37°C for 2 hours and the reaction is stopped by adding 20  $\mu$ l 1N NaOH per well. The absorbance is read at 610 nm. To calculate the amount of  $H_2O_2$  produced by the macrophages, a standard curve of a  $H_2O_2$  solution of known molarity is performed for each experiment.

25

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polypeptides, polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

5

### **Example 35: Biological Effects of Polypeptides of the Invention**

#### **Astrocyte and Neuronal Assays**

Recombinant polypeptides of the invention, expressed in *Escherichia coli* and purified as described above, can be tested for activity in promoting the survival, neurite  
10 outgrowth, or phenotypic differentiation of cortical neuronal cells and for inducing the proliferation of glial fibrillary acidic protein immunopositive cells, astrocytes. The selection of cortical cells for the bioassay is based on the prevalent expression of FGF-1 and FGF-2 in cortical structures and on the previously reported enhancement of cortical neuronal survival resulting from FGF-2 treatment. A thymidine incorporation assay, for  
15 example, can be used to elucidate a polypeptide of the invention's activity on these cells.

Moreover, previous reports describing the biological effects of FGF-2 (basic FGF) on cortical or hippocampal neurons *in vitro* have demonstrated increases in both neuron survival and neurite outgrowth (Walicke et al., "Fibroblast growth factor promotes survival of dissociated hippocampal neurons and enhances neurite extension." *Proc. Natl. Acad. Sci. USA* 83:3012-3016. (1986), assay herein incorporated by reference in its  
20 entirety). However, reports from experiments done on PC-12 cells suggest that these two responses are not necessarily synonymous and may depend on not only which FGF is being tested but also on which receptor(s) are expressed on the target cells. Using the primary cortical neuronal culture paradigm, the ability of a polypeptide of the invention to  
25 induce neurite outgrowth can be compared to the response achieved with FGF-2 using, for example, a thymidine incorporation assay.

Fibroblast and endothelial cell assays:

Human lung fibroblasts are obtained from Clonetics (San Diego, CA) and maintained in growth media from Clonetics. Dermal microvascular endothelial cells are obtained from Cell Applications (San Diego, CA). For proliferation assays, the human lung fibroblasts and dermal microvascular endothelial cells can be cultured at 5,000 cells/well in a 96-well plate for one day in growth medium. The cells are then incubated for one day in 0.1% BSA basal medium. After replacing the medium with fresh 0.1% BSA medium, the cells are incubated with the test proteins for 3 days. Alamar Blue (Alamar Biosciences, Sacramento, CA) is added to each well to a final concentration of 10%. The cells are incubated for 4 hr. Cell viability is measured by reading in a CytoFluor fluorescence reader. For the PGE<sub>2</sub> assays, the human lung fibroblasts are cultured at 5,000 cells/well in a 96-well plate for one day. After a medium change to 0.1% BSA basal medium, the cells are incubated with FGF-2 or polypeptides of the invention with or without IL-1 $\alpha$  for 24 hours. The supernatants are collected and assayed for PGE<sub>2</sub> by EIA kit (Cayman, Ann Arbor, MI). For the IL-6 assays, the human lung fibroblasts are cultured at 5,000 cells/well in a 96-well plate for one day. After a medium change to 0.1% BSA basal medium, the cells are incubated with FGF-2 or with or without polypeptides of the invention IL-1 $\alpha$  for 24 hours. The supernatants are collected and assayed for IL-6 by ELISA kit (Endogen, Cambridge, MA).

Human lung fibroblasts are cultured with FGF-2 or polypeptides of the invention for 3 days in basal medium before the addition of Alamar Blue to assess effects on growth of the fibroblasts. FGF-2 should show a stimulation at 10 - 2500 ng/ml which can be used to compare stimulation with polypeptides of the invention.

Parkinson Models.

The loss of motor function in Parkinson's disease is attributed to a deficiency of striatal dopamine resulting from the degeneration of the nigrostriatal dopaminergic projection neurons. An animal model for Parkinson's that has been extensively  
5 characterized involves the systemic administration of 1-methyl-4 phenyl 1,2,3,6-tetrahydropyridine (MPTP). In the CNS, MPTP is taken-up by astrocytes and catabolized by monoamine oxidase B to 1-methyl-4-phenyl pyridine (MPP<sup>+</sup>) and released. Subsequently, MPP<sup>+</sup> is actively accumulated in dopaminergic neurons by the high-affinity reuptake transporter for dopamine. MPP<sup>+</sup> is then concentrated in mitochondria by the  
10 electrochemical gradient and selectively inhibits nicotinamide adenine disphosphate: ubiquinone oxidoreductionase (complex I), thereby interfering with electron transport and eventually generating oxygen radicals.

It has been demonstrated in tissue culture paradigms that FGF-2 (basic FGF) has trophic activity towards nigral dopaminergic neurons (Ferrari et al., Dev. Biol. 1989).  
15 Recently, Dr. Unsicker's group has demonstrated that administering FGF-2 in gel foam implants in the striatum results in the near complete protection of nigral dopaminergic neurons from the toxicity associated with MPTP exposure (Otto and Unsicker, J. Neuroscience, 1990).

Based on the data with FGF-2, polypeptides of the invention can be evaluated to  
20 determine whether it has an action similar to that of FGF-2 in enhancing dopaminergic neuronal survival *in vitro* and it can also be tested *in vivo* for protection of dopaminergic neurons in the striatum from the damage associated with MPTP treatment. The potential effect of a polypeptide of the invention is first examined *in vitro* in a dopaminergic neuronal cell culture paradigm. The cultures are prepared by dissecting the midbrain floor  
25 plate from gestation day 14 Wistar rat embryos. The tissue is dissociated with trypsin and seeded at a density of 200,000 cells/cm<sup>2</sup> on polyorthinine-laminin coated glass coverslips.

The cells are maintained in Dulbecco's Modified Eagle's medium and F12 medium containing hormonal supplements (N1). The cultures are fixed with paraformaldehyde after 8 days *in vitro* and are processed for tyrosine hydroxylase, a specific marker for dopaminergic neurons, immunohistochemical staining. Dissociated cell cultures are prepared from embryonic rats. The culture medium is changed every third day and the factors are also added at that time.

Since the dopaminergic neurons are isolated from animals at gestation day 14, a developmental time which is past the stage when the dopaminergic precursor cells are proliferating, an increase in the number of tyrosine hydroxylase immunopositive neurons would represent an increase in the number of dopaminergic neurons surviving *in vitro*. Therefore, if a polypeptide of the invention acts to prolong the survival of dopaminergic neurons, it would suggest that the polypeptide may be involved in Parkinson's Disease.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

#### **Example 36: The Effect of Polypeptides of the Invention on the Growth of Vascular Endothelial Cells**

On day 1, human umbilical vein endothelial cells (HUVEC) are seeded at  $2-5 \times 10^4$  cells/35 mm dish density in M199 medium containing 4% fetal bovine serum (FBS), 16 units/ml heparin, and 50 units/ml endothelial cell growth supplements (ECGS, Biotechnology, Inc.). On day 2, the medium is replaced with M199 containing 10% FBS, 8 units/ml heparin. A polypeptide having the amino acid sequence of SEQ ID NO:Y, and positive controls, such as VEGF and basic FGF (bFGF) are added, at varying



concentrations. On days 4 and 6, the medium is replaced. On day 8, cell number is determined with a Coulter Counter.

An increase in the number of HUVEC cells indicates that the polypeptide of the invention may proliferate vascular endothelial cells.

- 5           The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

10   **Example 37: Stimulatory Effect of Polypeptides of the Invention on the Proliferation of Vascular Endothelial Cells**

- For evaluation of mitogenic activity of growth factors, the colorimetric MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)2H-tetrazolium) assay with the electron coupling reagent PMS (phenazine methosulfate) was performed (CellTiter 96 AQ, Promega). Cells are seeded in a 96-well plate (5,000 cells/well) in 0.1 mL serum-supplemented medium and are allowed to attach overnight. After serum-starvation for 12 hours in 0.5% FBS, conditions (bFGF, VEGF<sub>165</sub> or a polypeptide of the invention in 0.5% FBS) with or without Heparin (8 U/ml) are added to wells for 48 hours. 20 mg of MTS/PMS mixture (1:0.05) are added per well and allowed to incubate for 1 hour at 37°C before measuring the absorbance at 490 nm in an ELISA plate reader. Background absorbance from control wells (some media, no cells) is subtracted, and seven wells are performed in parallel for each condition. See, Leak *et al. In Vitro Cell. Dev. Biol.* 30A:512-518 (1994).

- 25           The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to

test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

**Example 38: Inhibition of PDGF-induced Vascular Smooth Muscle Cell**

**5 Proliferation Stimulatory Effect**

HAoSMC proliferation can be measured, for example, by BrdUrd incorporation. Briefly, subconfluent, quiescent cells grown on the 4-chamber slides are transfected with CRP or FITC-labeled AT2-3LP. Then, the cells are pulsed with 10% calf serum and 6  
10 mg/ml BrdUrd. After 24 h, immunocytochemistry is performed by using BrdUrd Staining Kit (Zymed Laboratories). In brief, the cells are incubated with the biotinylated mouse anti-BrdUrd antibody at 4 degrees C for 2 h after being exposed to denaturing solution and then incubated with the streptavidin-peroxidase and diaminobenzidine. After counterstaining with hematoxylin, the cells are mounted for microscopic examination, and  
15 the BrdUrd-positive cells are counted. The BrdUrd index is calculated as a percent of the BrdUrd-positive cells to the total cell number. In addition, the simultaneous detection of the BrdUrd staining (nucleus) and the FITC uptake (cytoplasm) is performed for individual cells by the concomitant use of bright field illumination and dark field-UV fluorescent illumination. See, Hayashida et al., J. Biol. Chem. 6:271(36):21985-21992  
20 (1996).

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

**Example 39: Stimulation of Endothelial Migration**

This example will be used to explore the possibility that a polypeptide of the invention may stimulate lymphatic endothelial cell migration.

5           Endothelial cell migration assays are performed using a 48 well microchemotaxis chamber (Neuroprobe Inc., Cabin John, MD; Falk, W., et al., J. Immunological Methods 1980;33:239-247). Polyvinylpyrrolidone-free polycarbonate filters with a pore size of 8 um (Nucleopore Corp. Cambridge, MA) are coated with 0.1% gelatin for at least 6 hours at room temperature and dried under sterile air. Test substances are diluted to appropriate  
10           concentrations in M199 supplemented with 0.25% bovine serum albumin (BSA), and 25 ul of the final dilution is placed in the lower chamber of the modified Boyden apparatus. Subconfluent, early passage (2-6) HUVEC or BMEC cultures are washed and trypsinized for the minimum time required to achieve cell detachment. After placing the filter between lower and upper chamber,  $2.5 \times 10^5$  cells suspended in 50 ul M199 containing 1%  
15           FBS are seeded in the upper compartment. The apparatus is then incubated for 5 hours at 37°C in a humidified chamber with 5% CO<sub>2</sub> to allow cell migration. After the incubation period, the filter is removed and the upper side of the filter with the non-migrated cells is scraped with a rubber policeman. The filters are fixed with methanol and stained with a Giemsa solution (Diff-Quick, Baxter, McGraw Park, IL). Migration is quantified by  
20           counting cells of three random high-power fields (40x) in each well, and all groups are performed in quadruplicate.

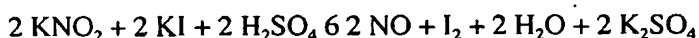
          The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the  
25           invention.

**Example 40: Stimulation of Nitric Oxide Production by Endothelial Cells**

Nitric oxide released by the vascular endothelium is believed to be a mediator of vascular endothelium relaxation. Thus, activity of a polypeptide of the invention can be  
 5 assayed by determining nitric oxide production by endothelial cells in response to the polypeptide.

Nitric oxide is measured in 96-well plates of confluent microvascular endothelial cells after 24 hours starvation and a subsequent 4 hr exposure to various levels of a positive control (such as VEGF-1) and the polypeptide of the invention. Nitric oxide in  
 10 the medium is determined by use of the Griess reagent to measure total nitrite after reduction of nitric oxide-derived nitrate by nitrate reductase. The effect of the polypeptide of the invention on nitric oxide release is examined on HUVEC.

Briefly, NO release from cultured HUVEC monolayer is measured with a NO-specific polarographic electrode connected to a NO meter (Iso-NO, World Precision  
 15 Instruments Inc.) (1049). Calibration of the NO elements is performed according to the following equation:



The standard calibration curve is obtained by adding graded concentrations of  $\text{KNO}_2$  (0, 5, 10, 25, 50, 100, 250, and 500 nmol/L) into the calibration solution containing  
 20 KI and  $\text{H}_2\text{SO}_4$ . The specificity of the Iso-NO electrode to NO is previously determined by measurement of NO from authentic NO gas (1050). The culture medium is removed and HUVECs are washed twice with Dulbecco's phosphate buffered saline. The cells are then bathed in 5 ml of filtered Krebs-Henseleit solution in 6-well plates, and the cell plates are kept on a slide warmer (Lab Line Instruments Inc.) To maintain the temperature at 37°C.  
 25 The NO sensor probe is inserted vertically into the wells, keeping the tip of the electrode 2 mm under the surface of the solution, before addition of the different conditions. S-nitroso acetyl penicillamin (SNAP) is used as a positive control. The amount of

released NO is expressed as picomoles per  $1 \times 10^6$  endothelial cells. All values reported are means of four to six measurements in each group (number of cell culture wells). See, Leak *et al. Biochem. and Biophys. Res. Comm.* 217:96-105 (1995).

The studies described in this example tested activity of polypeptides of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

**Example 41: Effect of Polypeptides of the Invention on Cord Formation in Angiogenesis**

Another step in angiogenesis is cord formation, marked by differentiation of endothelial cells. This bioassay measures the ability of microvascular endothelial cells to form capillary-like structures (hollow structures) when cultured *in vitro*.

CADMEC (microvascular endothelial cells) are purchased from Cell Applications, Inc. as proliferating (passage 2) cells and are cultured in Cell Applications' CADMEC Growth Medium and used at passage 5. For the *in vitro* angiogenesis assay, the wells of a 48-well cell culture plate are coated with Cell Applications' Attachment Factor Medium (200 ml/well) for 30 min. at 37°C. CADMEC are seeded onto the coated wells at 7,500 cells/well and cultured overnight in Growth Medium. The Growth Medium is then replaced with 300 mg Cell Applications' Chord Formation Medium containing control buffer or a polypeptide of the invention (0.1 to 100 ng/ml) and the cells are cultured for an additional 48 hr. The numbers and lengths of the capillary-like chords are quantitated through use of the Boeckeler VIA-170 video image analyzer. All assays are done in triplicate.

Commercial (R&D) VEGF (50 ng/ml) is used as a positive control. b-esteradiol (1 ng/ml) is used as a negative control. The appropriate buffer (without protein) is also utilized as a control.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

#### **Example 42: Angiogenic Effect on Chick Chorioallantoic Membrane**

10

Chick chorioallantoic membrane (CAM) is a well-established system to examine angiogenesis. Blood vessel formation on CAM is easily visible and quantifiable. The ability of polypeptides of the invention to stimulate angiogenesis in CAM can be examined.

15

Fertilized eggs of the White Leghorn chick (*Gallus gallus*) and the Japanese qual (*Coturnix coturnix*) are incubated at 37.8°C and 80% humidity. Differentiated CAM of 16-day-old chick and 13-day-old qual embryos is studied with the following methods.

20

On Day 4 of development, a window is made into the egg shell of chick eggs. The embryos are checked for normal development and the eggs sealed with cellotape. They are further incubated until Day 13. Thermanox coverslips (Nunc, Naperville, IL) are cut into disks of about 5 mm in diameter. Sterile and salt-free growth factors are dissolved in distilled water and about 3.3 mg/ 5 ml are pipetted on the disks. After air-drying, the inverted disks are applied on CAM. After 3 days, the specimens are fixed in 3% glutaraldehyde and 2% formaldehyde and rinsed in 0.12 M sodium cacodylate buffer.

25

They are photographed with a stereo microscope [Wild M8] and embedded for semi- and ultrathin sectioning as described above. Controls are performed with carrier disks alone.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

5

**Example 43: Angiogenesis Assay Using a Matrigel Implant in Mouse**

*In vivo* angiogenesis assay of a polypeptide of the invention measures the ability of an existing capillary network to form new vessels in an implanted capsule of murine extracellular matrix material (Matrigel). The protein is mixed with the liquid Matrigel at 4 degree C and the mixture is then injected subcutaneously in mice where it solidifies. After 10 7 days, the solid "plug" of Matrigel is removed and examined for the presence of new blood vessels. Matrigel is purchased from Becton Dickinson Labware/Collaborative Biomedical Products.

15 When thawed at 4 degree C the Matrigel material is a liquid. The Matrigel is mixed with a polypeptide of the invention at 150 ng/ml at 4 degrees C and drawn into cold 3 ml syringes. Female C57Bl/6 mice approximately 8 weeks old are injected with the mixture of Matrigel and experimental protein at 2 sites at the midventral aspect of the abdomen (0.5 ml/site). After 7 days, the mice are sacrificed by cervical dislocation, the 20 Matrigel plugs are removed and cleaned (i.e., all clinging membranes and fibrous tissue is removed). Replicate whole plugs are fixed in neutral buffered 10% formaldehyde, embedded in paraffin and used to produce sections for histological examination after staining with Masson's Trichrome. Cross sections from 3 different regions of each plug are processed. Selected sections are stained for the presence of vWF. The positive control 25 for this assay is bovine basic FGF (150 ng/ml). Matrigel alone is used to determine basal levels of angiogenesis.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

5

**Example 44: Rescue of Ischemia in Rabbit Lower Limb Model**

To study the in vivo effects of polynucleotides and polypeptides of the invention on ischemia, a rabbit hindlimb ischemia model is created by surgical removal of one femoral arteries as described previously (Takeshita *et al.*, *Am J. Pathol* 147:1649-1660 (1995)). The excision of the femoral artery results in retrograde propagation of thrombus and occlusion of the external iliac artery. Consequently, blood flow to the ischemic limb is dependent upon collateral vessels originating from the internal iliac artery (Takeshita *et al.* *Am J. Pathol* 147:1649-1660 (1995)). An interval of 10 days is allowed for post-operative recovery of rabbits and development of endogenous collateral vessels. At 10 day post-operatively (day 0), after performing a baseline angiogram, the internal iliac artery of the ischemic limb is transfected with 500 mg naked expression plasmid containing a polynucleotide of the invention by arterial gene transfer technology using a hydrogel-coated balloon catheter as described (Riessen *et al.* *Hum Gene Ther.* 4:749-758 (1993); Leclerc *et al.* *J. Clin. Invest.* 90: 936-944 (1992)). When a polypeptide of the invention is used in the treatment, a single bolus of 500 mg polypeptide of the invention or control is delivered into the internal iliac artery of the ischemic limb over a period of 1 min. through an infusion catheter. On day 30, various parameters are measured in these rabbits: (a) BP ratio - The blood pressure ratio of systolic pressure of the ischemic limb to that of normal limb; (b) Blood Flow and Flow Reserve - Resting FL: the blood flow during undilated condition and Max FL: the blood flow during fully dilated condition (also an indirect measure of the blood vessel amount) and Flow Reserve is reflected by the ratio



of max FL: resting FL; (c) Angiographic Score - This is measured by the angiogram of collateral vessels. A score is determined by the percentage of circles in an overlaying grid that with crossing opacified arteries divided by the total number in the rabbit thigh; (d) Capillary density - The number of collateral capillaries determined in light microscopic sections taken from hindlimbs.

The studies described in this example tested activity of polynucleotides and polypeptides of the invention. However, one skilled in the art could easily modify the exemplified studies to test the agonists, and/or antagonists of the invention.

#### 10 **Example 45: Effect of Polypeptides of the Invention on Vasodilation**

Since dilation of vascular endothelium is important in reducing blood pressure, the ability of polypeptides of the invention to affect the blood pressure in spontaneously hypertensive rats (SHR) is examined. Increasing doses (0, 10, 30, 100, 300, and 900 mg/kg) of the polypeptides of the invention are administered to 13-14 week old spontaneously hypertensive rats (SHR). Data are expressed as the mean +/- SEM. Statistical analysis are performed with a paired t-test and statistical significance is defined as  $p < 0.05$  vs. the response to buffer alone.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

#### 25 **Example 46: Rat Ischemic Skin Flap Model**

The evaluation parameters include skin blood flow, skin temperature, and factor VIII immunohistochemistry or endothelial alkaline phosphatase reaction. Expression of

polypeptides of the invention, during the skin ischemia, is studied using in situ hybridization.

The study in this model is divided into three parts as follows:

- a) Ischemic skin
- 5        b) Ischemic skin wounds
- c) Normal wounds

The experimental protocol includes:

- a) Raising a 3x4 cm, single pedicle full-thickness random skin flap (myocutaneous flap over the lower back of the animal).
- 10        b) An excisional wounding (4-6 mm in diameter) in the ischemic skin (skin-flap).
- c) Topical treatment with a polypeptide of the invention of the excisional wounds (day 0, 1, 2, 3, 4 post-wounding) at the following various dosage ranges: 1mg to 100 mg.
- d) Harvesting the wound tissues at day 3, 5, 7, 10, 14 and 21 post-wounding for histological, immunohistochemical, and in situ studies.

- 15        The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

20        **Example 47: Peripheral Arterial Disease Model**

Angiogenic therapy using a polypeptide of the invention is a novel therapeutic strategy to obtain restoration of blood flow around the ischemia in case of peripheral arterial diseases. The experimental protocol includes:

- 25        a) One side of the femoral artery is ligated to create ischemic muscle of the hindlimb, the other side of hindlimb serves as a control.

b) a polypeptide of the invention, in a dosage range of 20 mg - 500 mg, is delivered intravenously and/or intramuscularly 3 times (perhaps more) per week for 2-3 weeks.

c) The ischemic muscle tissue is collected after ligation of the femoral artery at 1, 2, and 3 weeks for the analysis of expression of a polypeptide of the invention and histology. Biopsy is also performed on the other side of normal muscle of the contralateral hindlimb.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

#### **Example 48: Ischemic Myocardial Disease Model**

A polypeptide of the invention is evaluated as a potent mitogen capable of stimulating the development of collateral vessels, and restructuring new vessels after coronary artery occlusion. Alteration of expression of the polypeptide is investigated in situ. The experimental protocol includes:

a) The heart is exposed through a left-side thoracotomy in the rat. Immediately, the left coronary artery is occluded with a thin suture (6-0) and the thorax is closed.

b) a polypeptide of the invention, in a dosage range of 20 mg - 500 mg, is delivered intravenously and/or intramuscularly 3 times (perhaps more) per week for 2-4 weeks.

c) Thirty days after the surgery, the heart is removed and cross-sectioned for morphometric and in situ analyzes.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to

test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

**Example 49: Rat Corneal Wound Healing Model**

5

This animal model shows the effect of a polypeptide of the invention on neovascularization. The experimental protocol includes:

- a) Making a 1-1.5 mm long incision from the center of cornea into the stromal layer.
- 10 b) Inserting a spatula below the lip of the incision facing the outer corner of the eye.
- c) Making a pocket (its base is 1-1.5 mm from the edge of the eye).
- d) Positioning a pellet, containing 50ng- 5ug of a polypeptide of the invention, within the pocket.
- 15 e) Treatment with a polypeptide of the invention can also be applied topically to the corneal wounds in a dosage range of 20mg - 500mg (daily treatment for five days).

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the  
20 invention.

**Example 50: Diabetic Mouse and Glucocorticoid-Impaired Wound Healing Models**

25 ***A. Diabetic db+/db+ Mouse Model.***

To demonstrate that a polypeptide of the invention accelerates the healing process, the genetically diabetic mouse model of wound healing is used. The full thickness wound

healing model in the db+/db+ mouse is a well characterized, clinically relevant and reproducible model of impaired wound healing. Healing of the diabetic wound is dependent on formation of granulation tissue and re-epithelialization rather than contraction (Gartner, M.H. *et al.*, *J. Surg. Res.* 52:389 (1992); Greenhalgh, D.G. *et al.*,  
5 *Am. J. Pathol.* 136:1235 (1990)).

The diabetic animals have many of the characteristic features observed in Type II diabetes mellitus. Homozygous (db+/db+) mice are obese in comparison to their normal heterozygous (db+/+m) littermates. Mutant diabetic (db+/db+) mice have a single autosomal recessive mutation on chromosome 4 (db+) (Coleman *et al. Proc. Natl. Acad.*  
10 *Sci. USA* 77:283-293 (1982)). Animals show polyphagia, polydipsia and polyuria. Mutant diabetic mice (db+/db+) have elevated blood glucose, increased or normal insulin levels, and suppressed cell-mediated immunity (Mandel *et al.*, *J. Immunol.* 120:1375 (1978); Debray-Sachs, M. *et al.*, *Clin. Exp. Immunol.* 51(1):1-7 (1983); Leiter *et al.*, *Am. J. of Pathol.* 114:46-55 (1985)). Peripheral neuropathy, myocardial complications, and  
15 microvascular lesions, basement membrane thickening and glomerular filtration abnormalities have been described in these animals (Norido, F. *et al.*, *Exp. Neurol.* 83(2):221-232 (1984); Robertson *et al.*, *Diabetes* 29(1):60-67 (1980); Giacomelli *et al.*, *Lab Invest.* 40(4):460-473 (1979); Coleman, D.L., *Diabetes* 31 (Suppl):1-6 (1982)). These homozygous diabetic mice develop hyperglycemia that is resistant to insulin analogous to  
20 human type II diabetes (Mandel *et al.*, *J. Immunol.* 120:1375-1377 (1978)).

The characteristics observed in these animals suggests that healing in this model may be similar to the healing observed in human diabetes (Greenhalgh, *et al.*, *Am. J. of Pathol.* 136:1235-1246 (1990)).

Genetically diabetic female C57BL/KsJ (db+/db+) mice and their non-diabetic  
25 (db+/+m) heterozygous littermates are used in this study (Jackson Laboratories). The animals are purchased at 6 weeks of age and are 8 weeks old at the beginning of the study.

Animals are individually housed and received food and water ad libitum. All manipulations are performed using aseptic techniques. The experiments are conducted according to the rules and guidelines of Human Genome Sciences, Inc. Institutional Animal Care and Use Committee and the Guidelines for the Care and Use of Laboratory  
5 Animals.

Wounding protocol is performed according to previously reported methods (Tsuboi, R. and Rifkin, D.B., *J. Exp. Med.* 172:245-251 (1990)). Briefly, on the day of wounding, animals are anesthetized with an intraperitoneal injection of Avertin (0.01 mg/mL), 2,2,2-tribromoethanol and 2-methyl-2-butanol dissolved in deionized water. The  
10 dorsal region of the animal is shaved and the skin washed with 70% ethanol solution and iodine. The surgical area is dried with sterile gauze prior to wounding. An 8 mm full-thickness wound is then created using a Keyes tissue punch. Immediately following wounding, the surrounding skin is gently stretched to eliminate wound expansion. The wounds are left open for the duration of the experiment. Application of the treatment is  
15 given topically for 5 consecutive days commencing on the day of wounding. Prior to treatment, wounds are gently cleansed with sterile saline and gauze sponges.

Wounds are visually examined and photographed at a fixed distance at the day of surgery and at two day intervals thereafter. Wound closure is determined by daily measurement on days 1-5 and on day 8. Wounds are measured horizontally and vertically  
20 using a calibrated Jameson caliper. Wounds are considered healed if granulation tissue is no longer visible and the wound is covered by a continuous epithelium.

A polypeptide of the invention is administered using at a range different doses, from 4mg to 500mg per wound per day for 8 days in vehicle. Vehicle control groups received 50mL of vehicle solution.

25 Animals are euthanized on day 8 with an intraperitoneal injection of sodium pentobarbital (300mg/kg). The wounds and surrounding skin are then harvested for

histology and immunohistochemistry. Tissue specimens are placed in 10% neutral buffered formalin in tissue cassettes between biopsy sponges for further processing.

Three groups of 10 animals each (5 diabetic and 5 non-diabetic controls) are evaluated: 1) Vehicle placebo control, 2) untreated group, and 3) treated group.

5        Wound closure is analyzed by measuring the area in the vertical and horizontal axis and obtaining the total square area of the wound. Contraction is then estimated by establishing the differences between the initial wound area (day 0) and that of post treatment (day 8). The wound area on day 1 is 64mm<sup>2</sup>, the corresponding size of the dermal punch. Calculations are made using the following formula:

10

$$[\text{Open area on day 8}] - [\text{Open area on day 1}] / [\text{Open area on day 1}]$$

Specimens are fixed in 10% buffered formalin and paraffin embedded blocks are sectioned perpendicular to the wound surface (5mm) and cut using a Reichert-Jung  
15        microtome. Routine hematoxylin-eosin (H&E) staining is performed on cross-sections of bisected wounds. Histologic examination of the wounds are used to assess whether the healing process and the morphologic appearance of the repaired skin is altered by treatment with a polypeptide of the invention. This assessment included verification of the presence of cell accumulation, inflammatory cells, capillaries, fibroblasts, re-  
20        epithelialization and epidermal maturity (Greenhalgh, D.G. *et al.*, *Am. J. Pathol.* 136:1235 (1990)). A calibrated lens micrometer is used by a blinded observer.

Tissue sections are also stained immunohistochemically with a polyclonal rabbit anti-human keratin antibody using ABC Elite detection system. Human skin is used as a positive tissue control while non-immune IgG is used as a negative control. Keratinocyte  
25        growth is determined by evaluating the extent of reepithelialization of the wound using a calibrated lens micrometer.

Proliferating cell nuclear antigen/cyclin (PCNA) in skin specimens is demonstrated by using anti-PCNA antibody (1:50) with an ABC Elite detection system. Human colon cancer can serve as a positive tissue control and human brain tissue can be used as a negative tissue control. Each specimen includes a section with omission of the primary  
5 antibody and substitution with non-immune mouse IgG. Ranking of these sections is based on the extent of proliferation on a scale of 0-8, the lower side of the scale reflecting slight proliferation to the higher side reflecting intense proliferation.

Experimental data are analyzed using an unpaired t test. A p value of < 0.05 is considered significant.

10

#### ***B. Steroid Impaired Rat Model***

The inhibition of wound healing by steroids has been well documented in various *in vitro* and *in vivo* systems (Wahl, Glucocorticoids and Wound healing. In: Anti-Inflammatory Steroid Action: Basic and Clinical Aspects. 280-302 (1989); Wahlet *et al.*, *J. Immunol.* 115: 476-481 (1975); Werb *et al.*, *J. Exp. Med.* 147:1684-1694 (1978)).  
15 Glucocorticoids retard wound healing by inhibiting angiogenesis, decreasing vascular permeability (Ebert *et al.*, *An. Intern. Med.* 37:701-705 (1952)), fibroblast proliferation, and collagen synthesis (Beck *et al.*, *Growth Factors.* 5: 295-304 (1991); Haynes *et al.*, *J. Clin. Invest.* 61: 703-797 (1978)) and producing a transient reduction of circulating  
20 monocytes (Haynes *et al.*, *J. Clin. Invest.* 61: 703-797 (1978); Wahl, "Glucocorticoids and wound healing", In: Antiinflammatory Steroid Action: Basic and Clinical Aspects, Academic Press, New York, pp. 280-302 (1989)). The systemic administration of steroids to impaired wound healing is a well establish phenomenon in rats (Beck *et al.*, *Growth Factors.* 5: 295-304 (1991); Haynes *et al.*, *J. Clin. Invest.* 61: 703-797 (1978); Wahl,  
25 "Glucocorticoids and wound healing", In: Antiinflammatory Steroid Action: Basic and



Clinical Aspects, Academic Press, New York, pp. 280-302 (1989); Pierce *et al.*, *Proc. Natl. Acad. Sci. USA* 86: 2229-2233 (1989)).

To demonstrate that a polypeptide of the invention can accelerate the healing process, the effects of multiple topical applications of the polypeptide on full thickness  
5 excisional skin wounds in rats in which healing has been impaired by the systemic administration of methylprednisolone is assessed.

Young adult male Sprague Dawley rats weighing 250-300 g (Charles River Laboratories) are used in this example. The animals are purchased at 8 weeks of age and are 9 weeks old at the beginning of the study. The healing response of rats is impaired by  
10 the systemic administration of methylprednisolone (17mg/kg/rat intramuscularly) at the time of wounding. Animals are individually housed and received food and water *ad libitum*. All manipulations are performed using aseptic techniques. This study is conducted according to the rules and guidelines of Human Genome Sciences, Inc. Institutional Animal Care and Use Committee and the Guidelines for the Care and Use of  
15 Laboratory Animals.

The wounding protocol is followed according to section A, above. On the day of wounding, animals are anesthetized with an intramuscular injection of ketamine (50 mg/kg) and xylazine (5 mg/kg). The dorsal region of the animal is shaved and the skin washed with 70% ethanol and iodine solutions. The surgical area is dried with sterile  
20 gauze prior to wounding. An 8 mm full-thickness wound is created using a Keyes tissue punch. The wounds are left open for the duration of the experiment. Applications of the testing materials are given topically once a day for 7 consecutive days commencing on the day of wounding and subsequent to methylprednisolone administration. Prior to treatment, wounds are gently cleansed with sterile saline and gauze sponges.

25 Wounds are visually examined and photographed at a fixed distance at the day of wounding and at the end of treatment. Wound closure is determined by daily measurement

on days 1-5 and on day 8. Wounds are measured horizontally and vertically using a calibrated Jameson caliper. Wounds are considered healed if granulation tissue is no longer visible and the wound is covered by a continuous epithelium.

The polypeptide of the invention is administered using at a range different doses, from 4mg to 500mg per wound per day for 8 days in vehicle. Vehicle control groups received 50mL of vehicle solution.

Animals are euthanized on day 8 with an intraperitoneal injection of sodium pentobarbital (300mg/kg). The wounds and surrounding skin are then harvested for histology. Tissue specimens are placed in 10% neutral buffered formalin in tissue cassettes between biopsy sponges for further processing.

Four groups of 10 animals each (5 with methylprednisolone and 5 without glucocorticoid) are evaluated: 1) Untreated group 2) Vehicle placebo control 3) treated groups.

Wound closure is analyzed by measuring the area in the vertical and horizontal axis and obtaining the total area of the wound. Closure is then estimated by establishing the differences between the initial wound area (day 0) and that of post treatment (day 8). The wound area on day 1 is 64mm<sup>2</sup>, the corresponding size of the dermal punch. Calculations are made using the following formula:

$$[\text{Open area on day 8}] - [\text{Open area on day 1}] / [\text{Open area on day 1}]$$

Specimens are fixed in 10% buffered formalin and paraffin embedded blocks are sectioned perpendicular to the wound surface (5mm) and cut using an Olympus microtome. Routine hematoxylin-eosin (H&E) staining is performed on cross-sections of bisected wounds. Histologic examination of the wounds allows assessment of whether the healing process and the morphologic appearance of the repaired skin is improved by

treatment with a polypeptide of the invention. A calibrated lens micrometer is used by a blinded observer to determine the distance of the wound gap.

Experimental data are analyzed using an unpaired t test. A p value of  $< 0.05$  is considered significant.

5        The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

#### 10    **Example 51: Lymphadema Animal Model**

or        The purpose of this experimental approach is to create an appropriate and consistent lymphedema model for testing the therapeutic effects of a polypeptide of the invention in lymphangiogenesis and re-establishment of the lymphatic circulatory system  
15    in the rat hind limb. Effectiveness is measured by swelling volume of the affected limb, quantification of the amount of lymphatic vasculature, total blood plasma protein, and histopathology. Acute lymphedema is observed for 7-10 days. Perhaps more importantly, the chronic progress of the edema is followed for up to 3-4 weeks.

      Prior to beginning surgery, blood sample is drawn for protein concentration  
20    analysis. Male rats weighing approximately ~350g are dosed with Pentobarbital. Subsequently, the right legs are shaved from knee to hip. The shaved area is swabbed with gauze soaked in 70% EtOH. Blood is drawn for serum total protein testing. Circumference and volumetric measurements are made prior to injecting dye into paws after marking 2 measurement levels (0.5 cm above heel, at mid-pt of dorsal paw). The  
25    intradermal dorsum of both right and left paws are injected with 0.05 ml of 1% Evan's Blue. Circumference and volumetric measurements are then made following injection of dye into paws.

Using the knee joint as a landmark, a mid-leg inguinal incision is made circumferentially allowing the femoral vessels to be located. Forceps and hemostats are used to dissect and separate the skin flaps. After locating the femoral vessels, the lymphatic vessel that runs along side and underneath the vessel(s) is located. The main  
5 lymphatic vessels in this area are then electrically coagulated suture ligated.

Using a microscope, muscles in back of the leg (near the semitendinosus and adductors) are bluntly dissected. The popliteal lymph node is then located. The 2 proximal and 2 distal lymphatic vessels and distal blood supply of the popliteal node are then and ligated by suturing. The popliteal lymph node, and any accompanying adipose  
10 tissue, is then removed by cutting connective tissues.

Care is taken to control any mild bleeding resulting from this procedure. After lymphatics are occluded, the skin flaps are sealed by using liquid skin (Vetbond) (AJ Buck). The separated skin edges are sealed to the underlying muscle tissue while leaving a gap of ~0.5 cm around the leg. Skin also may be anchored by suturing to underlying  
15 muscle when necessary.

To avoid infection, animals are housed individually with mesh (no bedding). Recovering animals are checked daily through the optimal edematous peak, which typically occurred by day 5-7. The plateau edematous peak are then observed. To evaluate the intensity of the lymphedema, the circumference and volumes of 2 designated  
20 places on each paw before operation and daily for 7 days are measured. The effect plasma proteins on lymphedema is determined and whether protein analysis is a useful testing perimeter is also investigated. The weights of both control and edematous limbs are evaluated at 2 places. Analysis is performed in a blind manner.

Circumference Measurements: Under brief gas anesthetic to prevent limb  
25 movement, a cloth tape is used to measure limb circumference. Measurements are done at

the ankle bone and dorsal paw by 2 different people then those 2 readings are averaged. Readings are taken from both control and edematous limbs.

Volumetric Measurements: On the day of surgery, animals are anesthetized with Pentobarbital and are tested prior to surgery. For daily volumetrics animals are under  
5 brief halothane anesthetic (rapid immobilization and quick recovery), both legs are shaved and equally marked using waterproof marker on legs. Legs are first dipped in water, then dipped into instrument to each marked level then measured by Buxco edema software(Chen/Victor). Data is recorded by one person, while the other is dipping the limb to marked area.

10 Blood-plasma protein measurements: Blood is drawn, spun, and serum separated prior to surgery and then at conclusion for total protein and Ca<sup>2+</sup> comparison.

Limb Weight Comparison: After drawing blood, the animal is prepared for tissue collection. The limbs are amputated using a quillitine, then both experimental and control legs are cut at the ligature and weighed. A second weighing is done as the tibio-cacaneal  
15 joint is disarticulated and the foot is weighed.

Histological Preparations: The transverse muscle located behind the knee (popliteal) area is dissected and arranged in a metal mold, filled with freezeGel, dipped into cold methylbutane, placed into labeled sample bags at - 80EC until sectioning. Upon sectioning, the muscle is observed under fluorescent microscopy for lymphatics..

20 The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

**Example 52: Suppression of TNF alpha-induced adhesion molecule expression by a Polypeptide of the Invention**

The recruitment of lymphocytes to areas of inflammation and angiogenesis involves specific receptor-ligand interactions between cell surface adhesion molecules (CAMs) on lymphocytes and the vascular endothelium. The adhesion process, in both normal and pathological settings, follows a multi-step cascade that involves intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and endothelial leukocyte adhesion molecule-1 (E-selectin) expression on endothelial cells (EC). The expression of these molecules and others on the vascular endothelium determines the efficiency with which leukocytes may adhere to the local vasculature and extravasate into the local tissue during the development of an inflammatory response. The local concentration of cytokines and growth factor participate in the modulation of the expression of these CAMs.

Tumor necrosis factor alpha (TNF- $\alpha$ ), a potent proinflammatory cytokine, is a stimulator of all three CAMs on endothelial cells and may be involved in a wide variety of inflammatory responses, often resulting in a pathological outcome.

The potential of a polypeptide of the invention to mediate a suppression of TNF- $\alpha$  induced CAM expression can be examined. A modified ELISA assay which uses ECs as a solid phase absorbent is employed to measure the amount of CAM expression on TNF- $\alpha$  treated ECs when co-stimulated with a member of the FGF family of proteins.

To perform the experiment, human umbilical vein endothelial cell (HUVEC) cultures are obtained from pooled cord harvests and maintained in growth medium (EGM-2; Clonetics, San Diego, CA) supplemented with 10% FCS and 1% penicillin/streptomycin in a 37 degree C humidified incubator containing 5% CO<sub>2</sub>. HUVECs are seeded in 96-well plates at concentrations of  $1 \times 10^4$  cells/well in EGM medium at 37 degree C for 18-24 hrs or until confluent. The monolayers are subsequently

washed 3 times with a serum-free solution of RPMI-1640 supplemented with 100 U/ml penicillin and 100 mg/ml streptomycin, and treated with a given cytokine and/or growth factor(s) for 24 h at 37 degree C. Following incubation, the cells are then evaluated for CAM expression.

- 5 Human Umbilical Vein Endothelial cells (HUVECs) are grown in a standard 96 well plate to confluence. Growth medium is removed from the cells and replaced with 90 ul of 199 Medium (10% FBS). Samples for testing and positive or negative controls are added to the plate in triplicate (in 10 ul volumes). Plates are incubated at 37 degree C for either 5 h (selectin and integrin expression) or 24 h (integrin expression only). Plates are  
10 aspirated to remove medium and 100  $\mu$ l of 0.1% paraformaldehyde-PBS(with Ca++ and Mg++) is added to each well. Plates are held at 4°C for 30 min.

- Fixative is then removed from the wells and wells are washed 1X with PBS(+Ca,Mg)+0.5% BSA and drained. Do not allow the wells to dry. Add 10  $\mu$ l of diluted primary antibody to the test and control wells. Anti-ICAM-1-Biotin, Anti-VCAM-  
15 1-Biotin and Anti-E-selectin-Biotin are used at a concentration of 10  $\mu$ g/ml (1:10 dilution of 0.1 mg/ml stock antibody). Cells are incubated at 37°C for 30 min. in a humidified environment. Wells are washed X3 with PBS(+Ca,Mg)+0.5% BSA.

- Then add 20  $\mu$ l of diluted ExtrAvidin-Alkaline Phosphatase (1:5,000 dilution) to each well and incubated at 37°C for 30 min. Wells are washed X3 with  
20 PBS(+Ca,Mg)+0.5% BSA. 1 tablet of p-Nitrophenol Phosphate pNPP is dissolved in 5 ml of glycine buffer (pH 10.4). 100  $\mu$ l of pNPP substrate in glycine buffer is added to each test well. Standard wells in triplicate are prepared from the working dilution of the ExtrAvidin-Alkaline Phosphatase in glycine buffer:  $1:5,000 (10^0) > 10^{-0.5} > 10^{-1} > 10^{-1.5}$ . 5  $\mu$ l of each dilution is added to triplicate wells and the resulting AP content in each well is  
25 5.50 ng, 1.74 ng, 0.55 ng, 0.18 ng. 100  $\mu$ l of pNNP reagent must then be added to each of the standard wells. The plate must be incubated at 37°C for 4h. A volume of 50  $\mu$ l of 3M

NaOH is added to all wells. The results are quantified on a plate reader at 405 nm. The background subtraction option is used on blank wells filled with glycine buffer only. The template is set up to indicate the concentration of AP-conjugate in each standard well [ 5.50 ng; 1.74 ng; 0.55 ng; 0.18 ng]. Results are indicated as amount of bound AP-conjugate in each sample.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

#### **Example 53: Assay for the Stimulation of Bone Marrow CD34+ Cell Proliferation**

This assay is based on the ability of human CD34+ to proliferate in the presence of hematopoietic growth factors and evaluates the ability of isolated polypeptides expressed in mammalian cells to stimulate proliferation of CD34+ cells.

It has been previously shown that most mature precursors will respond to only a single signal. More immature precursors require at least two signals to respond. Therefore, to test the effect of polypeptides on hematopoietic activity of a wide range of progenitor cells, the assay contains a given polypeptide in the presence or absence of other hematopoietic growth factors. Isolated cells are cultured for 5 days in the presence of Stem Cell Factor (SCF) in combination with tested sample. SCF alone has a very limited effect on the proliferation of bone marrow (BM) cells, acting in such conditions only as a "survival" factor. However, combined with any factor exhibiting stimulatory effect on these cells (e.g., IL-3), SCF will cause a synergistic effect. Therefore, if the tested polypeptide has a stimulatory effect on a hematopoietic progenitors, such activity can be easily detected. Since normal BM cells have a low



level of cycling cells, it is likely that any inhibitory effect of a given polypeptide, or agonists or antagonists thereof, might not be detected. Accordingly, assays for an inhibitory effect on progenitors is preferably tested in cells that are first subjected to *in vitro* stimulation with SCF+IL+3, and then contacted with the compound that is  
5 being evaluated for inhibition of such induced proliferation.

Briefly, CD34+ cells are isolated using methods known in the art. The cells are thawed and resuspended in medium (QBSF 60 serum-free medium with 1% L-glutamine (500ml) Quality Biological, Inc., Gaithersburg, MD Cat# 160-204-101). After several gentle centrifugation steps at 200 x g, cells are allowed to rest for one  
10 hour. The cell count is adjusted to  $2.5 \times 10^5$  cells/ml. During this time, 100  $\mu$ l of sterile water is added to the peripheral wells of a 96-well plate. The cytokines that can be tested with a given polypeptide in this assay is rhSCF (R&D Systems, Minneapolis, MN, Cat# 255-SC) at 50 ng/ml alone and in combination with rhSCF and rhIL-3 (R&D Systems, Minneapolis, MN, Cat# 203-ML) at 30 ng/ml. After one  
15 hour, 10  $\mu$ l of prepared cytokines, 50  $\mu$ l SID (supernatants at 1:2 dilution = 50  $\mu$ l) and 20  $\mu$ l of diluted cells are added to the media which is already present in the wells to allow for a final total volume of 100  $\mu$ l. The plates are then placed in a 37°C/5% CO<sub>2</sub> incubator for five days.

Eighteen hours before the assay is harvested, 0.5  $\mu$ Ci/well of [3H] Thymidine  
20 is added in a 10  $\mu$ l volume to each well to determine the proliferation rate. The experiment is terminated by harvesting the cells from each 96-well plate to a filtermat using the Tomtec Harvester 96. After harvesting, the filtermats are dried, trimmed and placed into OmniFilter assemblies consisting of one OmniFilter plate and one OmniFilter Tray. 60  $\mu$ l Microscint is added to each well and the plate sealed with  
25 TopSeal-A press-on sealing film. A bar code 15 sticker is affixed to the first plate for counting. The sealed plates is then loaded and the level of radioactivity determined

via the Packard Top Count and the printed data collected for analysis. The level of radioactivity reflects the amount of cell proliferation.

The studies described in this example test the activity of a given polypeptide to stimulate bone marrow CD34+ cell proliferation. One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), antibodies, agonists, and/or antagonists and fragments and variants thereof. As a nonlimiting example, potential antagonists tested in this assay would be expected to inhibit cell proliferation in the presence of cytokines and/or to increase the inhibition of cell proliferation in the presence of cytokines and a given polypeptide. In contrast, potential agonists tested in this assay would be expected to enhance cell proliferation and/or to decrease the inhibition of cell proliferation in the presence of cytokines and a given polypeptide.

The ability of a gene to stimulate the proliferation of bone marrow CD34+ cells indicates that polynucleotides and polypeptides corresponding to the gene are useful for the diagnosis and treatment of disorders affecting the immune system and hematopoiesis. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections above, and elsewhere herein.

**Example 54: Assay for Extracellular Matrix Enhanced Cell Response (EMECCR)**

The objective of the Extracellular Matrix Enhanced Cell Response (EMECCR) assay is to identify gene products (e.g., isolated polypeptides) that act on the hematopoietic stem cells in the context of the extracellular matrix (ECM) induced signal.

Cells respond to the regulatory factors in the context of signal(s) received from the surrounding microenvironment. For example, fibroblasts, and endothelial and epithelial stem cells fail to replicate in the absence of signals from the ECM. Hematopoietic stem cells can undergo self-renewal in the bone marrow, but not in *in*

*vitro* suspension culture. The ability of stem cells to undergo self-renewal *in vitro* is dependent upon their interaction with the stromal cells and the ECM protein fibronectin (fn). Adhesion of cells to fn is mediated by the  $\alpha_5\beta_1$  and  $\alpha_4\beta_1$  integrin receptors, which are expressed by human and mouse hematopoietic stem cells. The factor(s) which integrate with the ECM environment and responsible for stimulating stem cell self-renewal has not yet been identified. Discovery of such factors should be of great interest in gene therapy and bone marrow transplant applications

Briefly, polystyrene, non tissue culture treated, 96-well plates are coated with fn fragment at a coating concentration of  $0.2 \mu\text{g}/\text{cm}^2$ . Mouse bone marrow cells are plated (1,000 cells/well) in 0.2 ml of serum-free medium. Cells cultured in the presence of IL-3 (5 ng/ml) + SCF (50 ng/ml) would serve as the positive control, conditions under which little self-renewal but pronounced differentiation of the stem cells is to be expected. Gene products are tested with appropriate negative controls in the presence and absence of SCF(5.0 ng/ml), where test factor supernates represent 10% of the total assay volume. The plated cells are then allowed to grow by incubating in a low oxygen environment (5%  $\text{CO}_2$ , 7%  $\text{O}_2$ , and 88%  $\text{N}_2$ ) tissue culture incubator for 7 days. The number of proliferating cells within the wells is then quantitated by measuring thymidine incorporation into cellular DNA. Verification of the positive hits in the assay will require phenotypic characterization of the cells, which can be accomplished by scaling up of the culture system and using appropriate antibody reagents against cell surface antigens and FACScan.

One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), antibodies, agonists, and/or antagonists and fragments and variants thereof.

If a particular gene product is found to be a stimulator of hematopoietic progenitors, polynucleotides and polypeptides corresponding to the gene may be

useful for the diagnosis and treatment of disorders affecting the immune system and hematopoiesis. Representative uses are described in the “Immune Activity” and “Infectious Disease” sections above, and elsewhere herein. The gene product may also be useful in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types.

Additionally, the polynucleotides and/or polypeptides of the gene of interest and/or agonists and/or antagonists thereof, may also be employed to inhibit the proliferation and differentiation of hematopoietic cells and therefore may be employed to protect bone marrow stem cells from chemotherapeutic agents during chemotherapy. This antiproliferative effect may allow administration of higher doses of chemotherapeutic agents and, therefore, more effective chemotherapeutic treatment.

Moreover, polynucleotides and polypeptides corresponding to the gene of interest may also be useful for the treatment and diagnosis of hematopoietic related disorders such as, for example, anemia, pancytopenia, leukopenia, thrombocytopenia or leukemia since stromal cells are important in the production of cells of hematopoietic lineages. The uses include bone marrow cell ex-vivo culture, bone marrow transplantation, bone marrow reconstitution, radiotherapy or chemotherapy of neoplasia.

20

**Example 55: Human Dermal Fibroblast and Aortic Smooth Muscle Cell Proliferation**

The polypeptide of interest is added to cultures of normal human dermal fibroblasts (NHDF) and human aortic smooth muscle cells (AoSMC) and two co-assays are performed with each sample. The first assay examines the effect of the polypeptide of interest on the proliferation of normal human dermal fibroblasts

25

(NHDF) or aortic smooth muscle cells (AoSMC). Aberrant growth of fibroblasts or smooth muscle cells is a part of several pathological processes, including fibrosis, and restenosis. The second assay examines IL6 production by both NHDF and SMC. IL6 production is an indication of functional activation. Activated cells will have

5 increased production of a number of cytokines and other factors, which can result in a proinflammatory or immunomodulatory outcome. Assays are run with and without co-TNF $\alpha$  stimulation, in order to check for costimulatory or inhibitory activity.

Briefly, on day 1, 96-well black plates are set up with 1000 cells/well (NHDF) or 2000 cells/well (AoSMC) in 100  $\mu$ l culture media. NHDF culture media contains:

10 Clonetics FB basal media, 1mg/ml hFGF, 5mg/ml insulin, 50mg/ml gentamycin, 2%FBS, while AoSMC culture media contains Clonetics SM basal media, 0.5  $\mu$ g/ml hEGF, 5mg/ml insulin, 1 $\mu$ g/ml hFGF, 50mg/ml gentamycin, 50  $\mu$ g/ml Amphotericin B, 5%FBS. After incubation @ 37°C for at least 4-5 hours culture media is aspirated and replaced with growth arrest media. Growth arrest media for NHDF contains

15 fibroblast basal media, 50mg/ml gentamycin, 2% FBS, while growth arrest media for AoSMC contains SM basal media, 50mg/ml gentamycin, 50 $\mu$ g/ml Amphotericin B, 0.4% FBS. Incubate at 37C until day 2.

On day 2, serial dilutions and templates of the polypeptide of interest are designed which should always include media controls and known-protein controls.

20 For both stimulation and inhibition experiments, proteins are diluted in growth arrest media. For inhibition experiments, TNF $\alpha$  is added to a final concentration of 2ng/ml (NHDF) or 5ng/ml (AoSMC). Then add 1/3 vol media containing controls or supernatants and incubate at 37C/5% CO $_2$  until day 5.

Transfer 60 $\mu$ l from each well to another labeled 96-well plate, cover with a

25 plate-sealer, and store at 4C until Day 6 (for IL6 ELISA). To the remaining 100  $\mu$ l in the cell culture plate, aseptically add Alamar Blue in an amount equal to 10% of the

culture volume (10 $\mu$ l). Return plates to incubator for 3 to 4 hours. Then measure fluorescence with excitation at 530nm and emission at 590nm using the CytoFluor. This yields the growth stimulation/inhibition data.

On day 5, the IL6 ELISA is performed by coating a 96 well plate with 50-100  
5 ul/well of Anti-Human IL6 Monoclonal antibody diluted in PBS, pH 7.4, incubate ON at room temperature.

On day 6, empty the plates into the sink and blot on paper towels. Prepare Assay Buffer containing PBS with 4% BSA. Block the plates with 200  $\mu$ l/well of Pierce Super Block blocking buffer in PBS for 1-2 hr and then wash plates with wash  
10 buffer (PBS, 0.05% Tween-20). Blot plates on paper towels. Then add 50  $\mu$ l/well of diluted Anti-Human IL-6 Monoclonal, Biotin-labeled antibody at 0.50 mg/ml. Make dilutions of IL-6 stock in media (30, 10, 3, 1, 0.3, 0 ng/ml). Add duplicate samples to top row of plate. Cover the plates and incubate for 2 hours at RT on shaker.

Wash plates with wash buffer and blot on paper towels. Dilute EU-labeled  
15 Streptavidin 1:1000 in Assay buffer, and add 100  $\mu$ l/well. Cover the plate and incubate 1 h at RT. Wash plates with wash buffer. Blot on paper towels.

Add 100  $\mu$ l/well of Enhancement Solution. Shake for 5 minutes. Read the plate on the Wallac DELFIA Fluorometer. Readings from triplicate samples in each assay were tabulated and averaged.

20 A positive result in this assay suggests AoSMC cell proliferation and that the gene product of interest may be involved in dermal fibroblast proliferation and/or smooth muscle cell proliferation. A positive result also suggests many potential uses of polypeptides, polynucleotides, agonists and/or antagonists of the gene/gene product of interest. For example, inflammation and immune responses, wound healing, and  
25 angiogenesis, as detailed throughout this specification. Particularly, polypeptides of the gene product and polynucleotides of the gene may be used in wound healing and

dermal regeneration, as well as the promotion of vasculogenesis, both of the blood vessels and lymphatics. The growth of vessels can be used in the treatment of, for example, cardiovascular diseases. Additionally, antagonists of polypeptides of the gene product and polynucleotides of the gene may be useful in treating diseases, disorders, and/or conditions which involve angiogenesis by acting as an anti-vascular (e.g., anti-angiogenesis). These diseases, disorders, and/or conditions are known in the art and/or are described herein, such as, for example, malignancies, solid tumors, benign tumors, for example hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas; arteriosclerotic plaques; ocular angiogenic diseases, for example, diabetic retinopathy, retinopathy of prematurity, macular degeneration, corneal graft rejection, neovascular glaucoma, retrolental fibroplasia, rubeosis, retinoblastoma, uveitis and Pterygia (abnormal blood vessel growth) of the eye; rheumatoid arthritis; psoriasis; delayed wound healing; endometriosis; vasculogenesis; granulations; hypertrophic scars (keloids); nonunion fractures; scleroderma; trachoma; vascular adhesions; myocardial angiogenesis; coronary collaterals; cerebral collaterals; arteriovenous malformations; ischemic limb angiogenesis; Osler-Webber Syndrome; plaque neovascularization; telangiectasia; hemophiliac joints; angiofibroma; fibromuscular dysplasia; wound granulation; Crohn's disease; and atherosclerosis. Moreover, antagonists of polypeptides of the gene product and polynucleotides of the gene may be useful in treating anti-hyperproliferative diseases and/or anti-inflammatory known in the art and/or described herein.

One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), antibodies, agonists, and/or antagonists and fragments and variants thereof.

**Example 56: Cellular Adhesion Molecule (CAM) Expression on Endothelial Cells**

The recruitment of lymphocytes to areas of inflammation and angiogenesis involves specific receptor-ligand interactions between cell surface adhesion molecules (CAMs) on lymphocytes and the vascular endothelium. The adhesion process, in both normal and pathological settings, follows a multi-step cascade that involves intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and endothelial leukocyte adhesion molecule-1 (E-selectin) expression on endothelial cells (EC). The expression of these molecules and others on the vascular endothelium determines the efficiency with which leukocytes may adhere to the local vasculature and extravasate into the local tissue during the development of an inflammatory response. The local concentration of cytokines and growth factor participate in the modulation of the expression of these CAMs.

Briefly, endothelial cells (e.g., Human Umbilical Vein Endothelial cells (HUVECs)) are grown in a standard 96 well plate to confluence, growth medium is removed from the cells and replaced with 100  $\mu$ l of 199 Medium (10% fetal bovine serum (FBS)). Samples for testing and positive or negative controls are added to the plate in triplicate (in 10  $\mu$ l volumes). Plates are then incubated at 37°C for either 5 h (selectin and integrin expression) or 24 h (integrin expression only). Plates are aspirated to remove medium and 100  $\mu$ l of 0.1% paraformaldehyde-PBS(with Ca++ and Mg++) is added to each well. Plates are held at 4°C for 30 min. Fixative is removed from the wells and wells are washed 1X with PBS(+Ca,Mg) + 0.5% BSA and drained. 10  $\mu$ l of diluted primary antibody is added to the test and control wells. Anti-ICAM-1-Biotin, Anti-VCAM-1-Biotin and Anti-E-selectin-Biotin are used at a concentration of 10  $\mu$ g/ml (1:10 dilution of 0.1 mg/ml stock antibody). Cells are incubated at 37°C for 30 min. in a humidified environment. Wells are washed three



times with PBS(+Ca,Mg) + 0.5% BSA. 20  $\mu$ l of diluted ExtrAvidin-Alkaline Phosphotase (1:5,000 dilution, referred to herein as the working dilution) are added to each well and incubated at 37°C for 30 min. Wells are washed three times with PBS(+Ca,Mg)+0.5% BSA. Dissolve 1 tablet of p-Nitrophenol Phosphate pNPP per 5  
5 ml of glycine buffer (pH 10.4). 100  $\mu$ l of pNPP substrate in glycine buffer is added to each test well. Standard wells in triplicate are prepared from the working dilution of the ExtrAvidin-Alkaline Phosphotase in glycine buffer: 1:5,000 ( $10^0$ ) >  $10^{-0.5}$  >  $10^{-1}$  >  $10^{-1.5}$ . 5  $\mu$ l of each dilution is added to triplicate wells and the resulting AP content in each well is 5.50 ng, 1.74 ng, 0.55 ng, 0.18 ng. 100  $\mu$ l of pNNP reagent is then  
10 added to each of the standard wells. The plate is incubated at 37°C for 4h. A volume of 50  $\mu$ l of 3M NaOH is added to all wells. The plate is read on a plate reader at 405 nm using the background subtraction option on blank wells filled with glycine buffer only. Additionally, the template is set up to indicate the concentration of AP-conjugate in each standard well [ 5.50 ng; 1.74 ng; 0.55 ng; 0.18 ng]. Results are  
15 indicated as amount of bound AP-conjugate in each sample.

#### **Example 57: Alamar Blue Endothelial Cells Proliferation Assay**

This assay may be used to quantitatively determine protein mediated inhibition of bFGF-induced proliferation of Bovine Lymphatic Endothelial Cells  
20 (LECs), Bovine Aortic Endothelial Cells (BAECs) or Human Microvascular Uterine Myometrial Cells (UTMECs). This assay incorporates a fluorometric growth indicator based on detection of metabolic activity. A standard Alamar Blue Proliferation Assay is prepared in EGM-2MV with 10 ng /ml of bFGF added as a source of endothelial cell stimulation. This assay may be used with a variety of  
25 endothelial cells with slight changes in growth medium and cell concentration. Dilutions of the protein batches to be tested are diluted as appropriate. Serum-free

medium (GIBCO SFM) without bFGF is used as a non-stimulated control and Angiostatin or TSP-1 are included as a known inhibitory controls.

Briefly, LEC, BAECs or UTMECs are seeded in growth media at a density of 5000 to 2000 cells/well in a 96 well plate and placed at 37-C overnight. After the  
5 overnight incubation of the cells, the growth media is removed and replaced with GIBCO EC-SFM. The cells are treated with the appropriate dilutions of the protein of interest or control protein sample(s) (prepared in SFM ) in triplicate wells with additional bFGF to a concentration of 10 ng/ ml. Once the cells have been treated with the samples, the plate(s) is/are placed back in the 37° C incubator for three days.  
10 After three days 10 ml of stock alamar blue (Biosource Cat# DAL1100) is added to each well and the plate(s) is/are placed back in the 37°C incubator for four hours. The plate(s) are then read at 530nm excitation and 590nm emission using the CytoFluor fluorescence reader. Direct output is recorded in relative fluorescence units.

Alamar blue is an oxidation-reduction indicator that both fluoresces and  
15 changes color in response to chemical reduction of growth medium resulting from cell growth. As cells grow in culture, innate metabolic activity results in a chemical reduction of the immediate surrounding environment. Reduction related to growth causes the indicator to change from oxidized (non-fluorescent blue) form to reduced (fluorescent red) form. i.e. stimulated proliferation will produce a stronger signal and  
20 inhibited proliferation will produce a weaker signal and the total signal is proportional to the total number of cells as well as their metabolic activity. The background level of activity is observed with the starvation medium alone. This is compared to the output observed from the positive control samples (bFGF in growth medium) and protein dilutions.

25

**Example 58: Detection of Inhibition of a Mixed Lymphocyte Reaction**

This assay can be used to detect and evaluate inhibition of a Mixed Lymphocyte Reaction (MLR) by gene products (e.g., isolated polypeptides). Inhibition of a MLR may be due to a direct effect on cell proliferation and viability, modulation of costimulatory molecules on interacting cells, modulation of  
5 adhesiveness between lymphocytes and accessory cells, or modulation of cytokine production by accessory cells. Multiple cells may be targeted by these polypeptides since the peripheral blood mononuclear fraction used in this assay includes T, B and natural killer lymphocytes, as well as monocytes and dendritic cells.

Polypeptides of interest found to inhibit the MLR may find application in  
10 diseases associated with lymphocyte and monocyte activation or proliferation. These include, but are not limited to, diseases such as asthma, arthritis, diabetes, inflammatory skin conditions, psoriasis, eczema, systemic lupus erythematosus, multiple sclerosis, glomerulonephritis, inflammatory bowel disease, crohn's disease, ulcerative colitis, arteriosclerosis, cirrhosis, graft vs. host disease, host vs. graft  
15 disease, hepatitis, leukemia and lymphoma.

Briefly, PBMCs from human donors are purified by density gradient centrifugation using Lymphocyte Separation Medium (LSM®, density 1.0770 g/ml, Organon Teknika Corporation, West Chester, PA). PBMCs from two donors are adjusted to  $2 \times 10^6$  cells/ml in RPMI-1640 (Life Technologies, Grand Island, NY)  
20 supplemented with 10% FCS and 2 mM glutamine. PBMCs from a third donor is adjusted to  $2 \times 10^5$  cells/ml. Fifty microliters of PBMCs from each donor is added to wells of a 96-well round bottom microtiter plate. Dilutions of test materials (50  $\mu$ l) is added in triplicate to microtiter wells. Test samples (of the protein of interest) are added for final dilution of 1:4; rhuIL-2 (R&D Systems, Minneapolis, MN, catalog  
25 number 202-IL) is added to a final concentration of 1  $\mu$ g/ml; anti-CD4 mAb (R&D Systems, clone 34930.11, catalog number MAB379) is added to a final concentration

of 10 µg/ml. Cells are cultured for 7-8 days at 37°C in 5% CO<sub>2</sub>, and 1 µCi of [<sup>3</sup>H] thymidine is added to wells for the last 16 hrs of culture. Cells are harvested and thymidine incorporation determined using a Packard TopCount. Data is expressed as the mean and standard deviation of triplicate determinations.

- 5           Samples of the protein of interest are screened in separate experiments and compared to the negative control treatment, anti-CD4 mAb, which inhibits proliferation of lymphocytes and the positive control treatment, IL-2 (either as recombinant material or supernatant), which enhances proliferation of lymphocytes.

- 10           One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), antibodies, agonists, and/or antagonists and fragments and variants thereof.

- 15           It will be clear that the invention may be practiced otherwise than as particularly described in the foregoing description and examples. Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the appended claims.

- 20           The entire disclosure of each document cited (including patents, patent applications, journal articles, abstracts, laboratory manuals, books, or other disclosures) in the Background of the Invention, Detailed Description, and Examples is hereby incorporated herein by reference. Further, the hard copy of the sequence listing submitted herewith and the corresponding computer readable form are both incorporated herein by reference in their entireties.

Applicant's or agent's file reference number	PZ039PCT	International application No.	UNASSIGNED
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## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>153</u> , line <u>N/A</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution American Type Culture Collection	
Address of depositary institution (including postal code and country) 10801 University Boulevard Manassas, Virginia 20110-2209 United States of America	
Date of deposit 21 March 2000	Accession Number PTA-1543
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
Europe  In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC).	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	
For receiving Office use only	For International Bureau use only
<input type="checkbox"/> This sheet was received with the international application	<input type="checkbox"/> This sheet was received by the International Bureau on:
Authorized officer	Authorized officer:

**ATCC Deposit No. PTA-1543****CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

**NORWAY**

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

**AUSTRALIA**

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

**FINLAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

**UNITED KINGDOM**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

**ATCC Deposit No.: PTA-1543**

**DENMARK**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

**SWEDEN**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

**NETHERLANDS**

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

Applicant's or agent's file reference number	PZ039PCT	International application No.	UNASSIGNED
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## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>153</u> , line <u>N/A</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution American Type Culture Collection	
Address of depositary institution (including postal code and country) 10801 University Boulevard Manassas, Virginia 20110-2209 United States of America	
Date of deposit 21 March 2000	Accession Number PTA-1544
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
Europe  In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC).	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	
For receiving Office use only	For International Bureau use only
<input type="checkbox"/> This sheet was received with the international application	<input type="checkbox"/> This sheet was received by the International Bureau on:
Authorized officer	Authorized officer



**ATCC Deposit No. PTA-1544****CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

**NORWAY**

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

**AUSTRALIA**

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

**FINLAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

**UNITED KINGDOM**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

**ATCC Deposit No.: PTA-1544**

## **DENMARK**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

## **SWEDEN**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

## **NETHERLANDS**

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

Applicant's or agent's file reference number	PZ039PCT	International application No.	UNASSIGNED
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## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page 154, line N/A	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution American Type Culture Collection	
Address of depositary institution (including postal code and country) 10801 University Boulevard Manassas, Virginia 20110-2209 United States of America	
Date of deposit 11 August 1999	Accession Number PTA-499
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
Europe  In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC).	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	

<b>For receiving Office use only</b> <input type="checkbox"/> This sheet was received with the international application  Authorized officer	<b>For International Bureau use only</b> <input type="checkbox"/> This sheet was received by the International Bureau on:  Authorized officer
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**ATCC Deposit No. PTA-499****CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

**NORWAY**

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

**AUSTRALIA**

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

**FINLAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

**UNITED KINGDOM**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

**ATCC Deposit No.: PTA-499**

## **DENMARK**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

## **SWEDEN**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

## **NETHERLANDS**

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

Applicant's or agent's file reference number	PZ039PCT	International application No.	UNASSIGNED
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## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>154</u> , line <u>N/A</u>	
B. IDENTIFICATION OF DEPOSIT <span style="float: right;">Further deposits are identified on an additional sheet <input type="checkbox"/></span>	
Name of depositary institution American Type Culture Collection	
Address of depositary institution (including postal code and country) 10801 University Boulevard Manassas, Virginia 20110-2209 United States of America	
Date of deposit  09 July 1999	Accession Number  PTA-322
C. ADDITIONAL INDICATIONS (leave blank if not applicable) <span style="float: right;">This information is continued on an additional sheet <input type="checkbox"/></span>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
Europe  In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC).	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	

<p style="text-align: center;">For receiving Office use only</p> <p><input type="checkbox"/> This sheet was received with the international application</p> <p>Authorized officer</p>	<p style="text-align: center;">For International Bureau use only</p> <p><input type="checkbox"/> This sheet was received by the International Bureau on:</p> <p>Authorized officer</p>
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**ATCC Deposit No. PTA-322****CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

**NORWAY**

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

**AUSTRALIA**

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

**FINLAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

**UNITED KINGDOM**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

**ATCC Deposit No.: PTA-322**

**DENMARK**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

**SWEDEN**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

**NETHERLANDS**

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.



Applicant's or agent's file reference number	PZ039PCT	International application No.	UNASSIGNED
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## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>155</u> , line <u>N/A</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution American Type Culture Collection	
Address of depositary institution (including postal code and country) 10801 University Boulevard Manassas, Virginia 20110-2209 United States of America	
Date of deposit 08 April 1999	Accession Number 203918
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
Europe  In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC).	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	
For receiving Office use only	For International Bureau use only
<input type="checkbox"/> This sheet was received with the international application	<input type="checkbox"/> This sheet was received by the International Bureau on:
Authorized officer	Authorized officer

**ATCC Deposit No. 203918****CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

**NORWAY**

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

**AUSTRALIA**

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

**FINLAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

**UNITED KINGDOM**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

**ATCC Deposit No.: 203918****DENMARK**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

**SWEDEN**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

**NETHERLANDS**

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

Applicant's or agent's file reference number	PZ039PCT	International application No.	UNASSIGNED
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## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>155</u> , line <u>N/A</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution <u>American Type Culture Collection</u>	
Address of depositary institution (including postal code and country) <u>10801 University Boulevard</u> <u>Manassas, Virginia 20110-2209</u> <u>United States of America</u>	
Date of deposit <u>01 June 1999</u>	Accession Number <u>PTA-163</u>
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
<u>Europe</u>	
In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC).	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	
For receiving Office use only	For International Bureau use only
<input type="checkbox"/> This sheet was received with the international application	<input type="checkbox"/> This sheet was received by the International Bureau on:
Authorized officer	Authorized officer

**ATCC Deposit No. PTA-163****CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

**NORWAY**

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

**AUSTRALIA**

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

**FINLAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

**UNITED KINGDOM**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

**ATCC Deposit No.: PTA-163**

**DENMARK**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

**SWEDEN**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

**NETHERLANDS**

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

Applicant's or agent's file reference number	PZ039PCT	International application No.	UNASSIGNED
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## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>156</u> , line <u>N/A</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution American Type Culture Collection	
Address of depositary institution (including postal code and country) 10801 University Boulevard Manassas, Virginia 20110-2209 United States of America	
Date of deposit 26 April 1999	Accession Number 203959
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
Europe  In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC).	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	
For receiving Office use only	For International Bureau use only
<input type="checkbox"/> This sheet was received with the international application	<input type="checkbox"/> This sheet was received by the International Bureau on:
Authorized officer	Authorized officer

**ATCC Deposit No. 203959****CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

**NORWAY**

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

**AUSTRALIA**

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

**FINLAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

**UNITED KINGDOM**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.



**ATCC Deposit No.: 203959**

**DENMARK**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

**SWEDEN**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

**NETHERLANDS**

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

Applicant's or agent's file reference number	P2039PCT	International application No.	UNASSIGNED
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## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>157</u> , line <u>N/A</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution American Type Culture Collection	
Address of depositary institution (including postal code and country) 10801 University Boulevard Manassas, Virginia 20110-2209 United States of America	
Date of deposit 27 September 1999	Accession Number PTA-794
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
Europe  In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC).	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	

For receiving Office use only	For International Bureau use only
<input type="checkbox"/> This sheet was received with the international application	<input type="checkbox"/> This sheet was received by the International Bureau on:
Authorized officer	Authorized officer

**ATCC Deposit No. PTA-794****CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

**NORWAY**

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

**AUSTRALIA**

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

**FINLAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

**UNITED KINGDOM**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

**ATCC Deposit No.: PTA-794**

## **DENMARK**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

## **SWEDEN**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

## **NETHERLANDS**

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

Applicant's or agent's file reference number	P2039PCT	International application No.	UNASSIGNED
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## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>157</u> , line <u>N/A</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution American Type Culture Collection	
Address of depositary institution (including postal code and country) 10801 University Boulevard Manassas, Virginia 20110-2209 United States of America	
Date of deposit 09 September 1998	Accession Number 203181
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
Europe  In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC).	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	
For receiving Office use only	For International Bureau use only
<input type="checkbox"/> This sheet was received with the international application	<input type="checkbox"/> This sheet was received by the International Bureau on:
Authorized officer	Authorized officer

**ATCC Deposit No. 203181****CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

**NORWAY**

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

**AUSTRALIA**

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

**FINLAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

**UNITED KINGDOM**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

**ATCC Deposit No.: 203181****DENMARK**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

**SWEDEN**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

**NETHERLANDS**

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

Applicant's or agent's file reference number	PZ039PCT	International application No.	UNASSIGNED
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## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>159</u> , line <u>N/A</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution American Type Culture Collection	
Address of depositary institution (including postal code and country) 10801 University Boulevard Manassas, Virginia 20110-2209 United States of America	
Date of deposit 04 March 1998	Accession Number 209651
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
Europe  In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC).	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g. "Accession Number of Deposit")	
For receiving Office use only	For International Bureau use only
<input type="checkbox"/> This sheet was received with the international application	<input type="checkbox"/> This sheet was received by the International Bureau on:
Authorized officer	Authorized officer



**ATCC Deposit No. 209651****CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

**NORWAY**

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

**AUSTRALIA**

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

**FINLAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

**UNITED KINGDOM**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

**ATCC Deposit No.: 209651**

**DENMARK**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

**SWEDEN**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

**NETHERLANDS**

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

Applicant's or agent's file reference number	PZ039PCT	International application No.	UNASSIGNED
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## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>159</u> , line <u>N/A</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution <u>American Type Culture Collection</u>	
Address of depositary institution (including postal code and country) <u>10801 University Boulevard</u> <u>Manassas, Virginia 20110-2209</u> <u>United States of America</u>	
Date of deposit <u>07 June 1999</u>	Accession Number <u>PTA-181</u>
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
<u>Europe</u>	
In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC).	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	

For receiving Office use only	For International Bureau use only
<input type="checkbox"/> This sheet was received with the international application	<input type="checkbox"/> This sheet was received by the International Bureau on:
Authorized officer	Authorized officer

**ATCC Deposit No. PTA-181****CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

**NORWAY**

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

**AUSTRALIA**

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

**FINLAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

**UNITED KINGDOM**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

**ATCC Deposit No.: PTA-181****DENMARK**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

**SWEDEN**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

**NETHERLANDS**

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

Applicant's or agent's file reference number	PZ039PCT	International application No.	UNASSIGNED
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## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>159</u> , line <u>N/A</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution American Type Culture Collection	
Address of depositary institution (including postal code and country) 10801 University Boulevard Manassas, Virginia 20110-2209 United States of America	
Date of deposit 01 August 1997	Accession Number 209195
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
Europe  In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC).	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	

For receiving Office use only
<input type="checkbox"/> This sheet was received with the international application
Authorized officer

For International Bureau use only
<input type="checkbox"/> This sheet was received by the International Bureau on:
Authorized officer

**ATCC Deposit No. 209195****CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

**NORWAY**

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

**AUSTRALIA**

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

**FINLAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

**UNITED KINGDOM**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

**ATCC Deposit No.: 209195**

**DENMARK**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

**SWEDEN**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

**NETHERLANDS**

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.



Applicant's or agent's file reference number	PZ039PCT	International application No.	UNASSIGNED
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## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>159</u> , line <u>N/A</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution American Type Culture Collection	
Address of depositary institution (including postal code and country) 10801 University Boulevard Manassas, Virginia 20110-2209 United States of America	
Date of deposit 07 April 1998	Accession Number 209746
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States) Europe  In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC).	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable) The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	
For receiving Office use only <input type="checkbox"/> This sheet was received with the international application  Authorized officer	For International Bureau use only <input type="checkbox"/> This sheet was received by the International Bureau on:  Authorized officer

**ATCC Deposit No. 209746****CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

**NORWAY**

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**AUSTRALIA**

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**FINLAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

**UNITED KINGDOM**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

**ATCC Deposit No.: 209746**

**DENMARK**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

**SWEDEN**

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**NETHERLANDS**

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

Applicant's or agent's file reference number	PZ039PCT	International application No.	UNASSIGNED
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## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>159</u> , line <u>N/A</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution American Type Culture Collection	
Address of depositary institution (including postal code and country) 10801 University Boulevard Manassas, Virginia 20110-2209 United States of America	
Date of deposit 02 November 1999	Accession Number PTA-909
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
Europe  In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC).	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	
For receiving Office use only	For International Bureau use only
<input type="checkbox"/> This sheet was received with the international application	<input type="checkbox"/> This sheet was received by the International Bureau on:
Authorized officer	Authorized officer

**ATCC Deposit No. PTA-909****CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

**NORWAY**

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**AUSTRALIA**

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**FINLAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

**UNITED KINGDOM**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

**ATCC Deposit No.: PTA-909****DENMARK**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

**SWEDEN**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

**NETHERLANDS**

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***What Is Claimed Is:***

1. An isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence at least 95% identical to a sequence selected from the group  
5 consisting of:
  - (a) a polynucleotide fragment of SEQ ID NO:X or a polynucleotide fragment of the cDNA sequence included in ATCC Deposit No:Z, which is hybridizable to SEQ ID NO:X;
  - (b) a polynucleotide encoding a polypeptide fragment of SEQ ID NO:Y or a  
10 polypeptide fragment encoded by the cDNA sequence included in ATCC Deposit No:Z, which is hybridizable to SEQ ID NO:X;
  - (c) a polynucleotide encoding a polypeptide domain of SEQ ID NO:Y or a polypeptide domain encoded by the cDNA sequence included in ATCC Deposit No:Z, which is hybridizable to SEQ ID NO:X;
  - 15 (d) a polynucleotide encoding a polypeptide epitope of SEQ ID NO:Y or a polypeptide epitope encoded by the cDNA sequence included in ATCC Deposit No:Z, which is hybridizable to SEQ ID NO:X;
  - (e) a polynucleotide encoding a polypeptide of SEQ ID NO:Y or the cDNA sequence included in ATCC Deposit No:Z, which is hybridizable to SEQ ID NO:X,  
20 having biological activity;
  - (f) a polynucleotide which is a variant of SEQ ID NO:X;
  - (g) a polynucleotide which is an allelic variant of SEQ ID NO:X;
  - (h) a polynucleotide which encodes a species homologue of the SEQ ID NO:Y;
  - 25 (i) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(h), wherein said polynucleotide does not

hybridize under stringent conditions to a nucleic acid molecule having a nucleotide sequence of only A residues or of only T residues.

2. The isolated nucleic acid molecule of claim 1, wherein the  
5 polynucleotide fragment comprises a nucleotide sequence encoding a secreted protein.

3. The isolated nucleic acid molecule of claim 1, wherein the  
polynucleotide fragment comprises a nucleotide sequence encoding the sequence  
10 identified as SEQ ID NO:Y or the polypeptide encoded by the cDNA sequence included in ATCC Deposit No:Z, which is hybridizable to SEQ ID NO:X.

4. The isolated nucleic acid molecule of claim 1, wherein the  
polynucleotide fragment comprises the entire nucleotide sequence of SEQ ID NO:X  
15 or the cDNA sequence included in ATCC Deposit No:Z, which is hybridizable to SEQ ID NO:X.

5. The isolated nucleic acid molecule of claim 2, wherein the nucleotide  
sequence comprises sequential nucleotide deletions from either the C-terminus or the  
20 N-terminus.

6. The isolated nucleic acid molecule of claim 3, wherein the nucleotide  
sequence comprises sequential nucleotide deletions from either the C-terminus or the  
N-terminus.



7. A recombinant vector comprising the isolated nucleic acid molecule of claim 1.

8. A method of making a recombinant host cell comprising the isolated  
5 nucleic acid molecule of claim 1.

9. A recombinant host cell produced by the method of claim 8.

10. The recombinant host cell of claim 9 comprising vector sequences.  
10

11. An isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence selected from the group consisting of:

(a) a polypeptide fragment of SEQ ID NO:Y or the encoded sequence included in ATCC Deposit No:Z;

15 (b) a polypeptide fragment of SEQ ID NO:Y or the encoded sequence included in ATCC Deposit No:Z, having biological activity;

(c) a polypeptide domain of SEQ ID NO:Y or the encoded sequence included in ATCC Deposit No:Z;

20 (d) a polypeptide epitope of SEQ ID NO:Y or the encoded sequence included in ATCC Deposit No:Z;

(e) a secreted form of SEQ ID NO:Y or the encoded sequence included in ATCC Deposit No:Z;

(f) a full length protein of SEQ ID NO:Y or the encoded sequence included in ATCC Deposit No:Z;

25 (g) a variant of SEQ ID NO:Y;

(h) an allelic variant of SEQ ID NO:Y; or

(i) a species homologue of the SEQ ID NO:Y.

12. The isolated polypeptide of claim 11, wherein the secreted form or the full length protein comprises sequential amino acid deletions from either the C-terminus or the N-terminus.

5

13. An isolated antibody that binds specifically to the isolated polypeptide of claim 11.

14. A recombinant host cell that expresses the isolated polypeptide of  
10 claim 11.

15. A method of making an isolated polypeptide comprising:

(a) culturing the recombinant host cell of claim 14 under conditions such that said polypeptide is expressed; and

15 (b) recovering said polypeptide.

16. The polypeptide produced by claim 15.

17. A method for preventing, treating, or ameliorating a medical condition,  
20 comprising administering to a mammalian subject a therapeutically effective amount of the polypeptide of claim 11 or the polynucleotide of claim 1.

18. A method of diagnosing a pathological condition or a susceptibility to a pathological condition in a subject comprising:

25 (a) determining the presence or absence of a mutation in the polynucleotide of claim 1; and

(b) diagnosing a pathological condition or a susceptibility to a pathological condition based on the presence or absence of said mutation.

19. A method of diagnosing a pathological condition or a susceptibility to  
5 a pathological condition in a subject comprising:

(a) determining the presence or amount of expression of the polypeptide of  
claim 11 in a biological sample; and

(b) diagnosing a pathological condition or a susceptibility to a pathological  
condition based on the presence or amount of expression of the polypeptide.

10

20. A method for identifying a binding partner to the polypeptide of claim  
11 comprising:

(a) contacting the polypeptide of claim 11 with a binding partner; and

(b) determining whether the binding partner effects an activity of the  
15 polypeptide.

21. The gene corresponding to the cDNA sequence of SEQ ID NO:Y.

22. A method of identifying an activity in a biological assay, wherein the  
20 method comprises:

(a) expressing SEQ ID NO:X in a cell;

(b) isolating the supernatant;

(c) detecting an activity in a biological assay; and

(d) identifying the protein in the supernatant having the activity.

25

23. The product produced by the method of claim 20.

<110> Human Genome Sciences, Inc.

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<130> PZ039PCT

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<141> 2000-04-06

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aaaaaaaaaa aaaaaaaaaa aaa                                     1763

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&lt;210&gt; 18

&lt;211&gt; 703

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 18

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&lt;210&gt; 19

&lt;211&gt; 774

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (760)

&lt;223&gt; n equals a,t,g, or c

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (763)

&lt;223&gt; n equals a,t,g, or c

&lt;400&gt; 19

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&lt;210&gt; 20

&lt;211&gt; 1549

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (873)

&lt;223&gt; n equals a,t,g, or c

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (895)

&lt;223&gt; n equals a,t,g, or c

&lt;400&gt; 20

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&lt;210&gt; 21

&lt;211&gt; 1189

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 21

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&lt;210&gt; 22

&lt;211&gt; 2460

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

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 <223> n equals a,t,g, or c

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 <221> SITE  
 <222> (2459)  
 <223> n equals a,t,g, or c

<220>  
 <221> SITE  
 <222> (2460)  
 <223> n equals a,t,g, or c

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aaccccgtag	aagtcggtcg	gcaggtcctc	tccaacccgc	cgctaccgcg	ccgctgtggg	240
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&lt;210&gt; 23

&lt;211&gt; 4386

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (3477)

&lt;223&gt; n equals a,t,g, or c

&lt;400&gt; 23

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&lt;210&gt; 24

&lt;211&gt; 2462

&lt;212&gt; DNA

<213> Homo sapiens

<220>

<221> SITE

<222> (10)

<223> n equals a,t,g, or c

<220>

<221> SITE

<222> (2461)

<223> n equals a,t,g, or c

<400> 24

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na						2462

&lt;210&gt; 25

&lt;211&gt; 2635

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 25

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&lt;210&gt; 26

&lt;211&gt; 2707

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 26

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&lt;210&gt; 27

&lt;211&gt; 1898

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (1398)

&lt;223&gt; n equals a,t,g, or c

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (1428)

&lt;223&gt; n equals a,t,g, or c

&lt;400&gt; 27

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&lt;210&gt; 28

&lt;211&gt; 2298

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (4)

&lt;223&gt; n equals a,t,g, or c

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (1653)

&lt;223&gt; n equals a,t,g, or c

&lt;400&gt; 28

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&lt;210&gt; 29

&lt;211&gt; 1481

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 29

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&lt;210&gt; 30

&lt;211&gt; 1012

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 30

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&lt;210&gt; 31

&lt;211&gt; 1886

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 31

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ccacctccaa	aaaaaaaaaa	aaaaaa				1886

&lt;210&gt; 32

&lt;211&gt; 2406

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (1934)

&lt;223&gt; n equals a,t,g, or c

&lt;400&gt; 32

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aaaaaa						2406

&lt;210&gt; 33

&lt;211&gt; 2636

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 33

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&lt;210&gt; 34

&lt;211&gt; 1461

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 34

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&lt;210&gt; 35

&lt;211&gt; 953

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 35

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&lt;210&gt; 36

&lt;211&gt; 1340

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (851)

&lt;223&gt; n equals a,t,g, or c

&lt;400&gt; 36

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&lt;210&gt; 37

&lt;211&gt; 2199

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 37

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27

<210> 38  
 <211> 989  
 <212> DNA  
 <213> Homo sapiens

<220>  
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 <222> (955)  
 <223> n equals a,t,g, or c

<220>  
 <221> SITE  
 <222> (979)  
 <223> n equals a,t,g, or c

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 <211> 2048  
 <212> DNA  
 <213> Homo sapiens

<400> 39  
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&lt;210&gt; 40

&lt;211&gt; 634

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 40

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&lt;210&gt; 41

&lt;211&gt; 2763

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 41

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&lt;210&gt; 42



&lt;211&gt; 655

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 42

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&lt;210&gt; 43

&lt;211&gt; 2410

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 43

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aaaaaaaaa						2410

&lt;210&gt; 44

&lt;211&gt; 2634

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 44

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&lt;210&gt; 45

&lt;211&gt; 448

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 45

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&lt;210&gt; 46

&lt;211&gt; 2921

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 46

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&lt;210&gt; 47

&lt;211&gt; 419

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (2)

&lt;223&gt; n equals a,t,g, or c

&lt;400&gt; 47

tnataataat gtctttttcca tcatgtctga ctttagtaat ttaagtcttc tcttttttct

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&lt;210&gt; 48

&lt;211&gt; 931

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (717)

&lt;223&gt; n equals a,t,g, or c

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (718)

&lt;223&gt; n equals a,t,g, or c

&lt;400&gt; 48

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&lt;210&gt; 49

&lt;211&gt; 760

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 49

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&lt;210&gt; 50

&lt;211&gt; 2479

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (240)

&lt;223&gt; n equals a,t,g, or c

&lt;400&gt; 50

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&lt;210&gt; 51

&lt;211&gt; 1573

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 51

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aaaaaaaaaa	aaa					1573

<210> 52  
 <211> 1677  
 <212> DNA  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (537)  
 <223> n equals a,t,g, or c

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 <212> DNA  
 <213> Homo sapiens

<400> 53  
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 gtactatagg taagttgggc ggaaatcctt gctgttatgt ttaatgtccc ttgccatttt 240



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&lt;210&gt; 54

&lt;211&gt; 1646

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (1544)

&lt;223&gt; n equals a,t,g, or c

&lt;400&gt; 54

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&lt;210&gt; 55

&lt;211&gt; 1558

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (1443)

&lt;223&gt; n equals a,t,g, or c

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (1460)

&lt;223&gt; n equals a,t,g, or c

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (1494)

&lt;223&gt; n equals a,t,g, or c

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (1537)

&lt;223&gt; n equals a,t,g, or c

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (1543)

&lt;223&gt; n equals a,t,g, or c

&lt;400&gt; 55

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&lt;210&gt; 56

&lt;211&gt; 753

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 56

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&lt;210&gt; 57

&lt;211&gt; 1769

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 57

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&lt;210&gt; 58

&lt;211&gt; 626

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 58

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taaaaaaaaa aaaaaaaaaa aaaagg	626

&lt;210&gt; 59

&lt;211&gt; 634

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 59

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tgtgcctggt gaccaaagtc cctccaaagt gctcttcctt ctgggttatt caagccaaat	360
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aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaa	634

&lt;210&gt; 60

&lt;211&gt; 627

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 60

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ctcttggcca gtccatgaa tgggccttag atgatgcccc tgaaattgca tgcaaatgt	120
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&lt;210&gt; 61

&lt;211&gt; 632

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 61

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&lt;210&gt; 62

&lt;211&gt; 706

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 62

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aagtaaaaaa	aaaaaaaaaa	aaaaaaaaaa	aaaaaaaaaa	aaaaaa		706

&lt;210&gt; 63

&lt;211&gt; 1345

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 63

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aatttgcaaa	aaaaaaaaaa	aaaaa				1345

&lt;210&gt; 64

&lt;211&gt; 773

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (3)

&lt;223&gt; n equals a,t,g, or c

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (11)

&lt;223&gt; n equals a,t,g, or c

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (51)

&lt;223&gt; n equals a,t,g, or c

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (53)

&lt;223&gt; n equals a,t,g, or c

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (69)

&lt;223&gt; n equals a,t,g, or c

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (112)

&lt;223&gt; n equals a,t,g, or c

&lt;400&gt; 64

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&lt;210&gt; 65

&lt;211&gt; 1569

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (282)

&lt;223&gt; n equals a,t,g, or c

&lt;400&gt; 65

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aaaaaaaaa						1569

&lt;210&gt; 66



<211> 2657  
 <212> DNA  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (179)  
 <223> n equals a,t,g, or c

<400> 66  
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 tatgtacagt tctctgtgtt aacagctgag aagtaagcaa ccttttctga ctgcataatg 180  
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 acagttgtct tattcgtatt gtttataaac tttgagggtt aggactgggt cttactcatc 300  
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<210> 67  
 <211> 1355  
 <212> DNA  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (1327)  
 <223> n equals a,t,g, or c

<400> 67	
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<210> 68  
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 <212> DNA  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (927)  
 <223> n equals a,t,g, or c

<220>  
 <221> SITE  
 <222> (929)  
 <223> n equals a,t,g, or c

<400> 68

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<210> 69  
 <211> 1799  
 <212> DNA  
 <213> Homo sapiens

<400> 69

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&lt;210&gt; 70

&lt;211&gt; 1984

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 70

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accg

1984

&lt;210&gt; 71

&lt;211&gt; 2084

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 71

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&lt;210&gt; 72

&lt;211&gt; 734

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 72

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aaaaaaaaaa	aaaa					734

&lt;210&gt; 73

&lt;211&gt; 4385

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (3476)

&lt;223&gt; n equals a,t,g, or c

&lt;400&gt; 73

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 <222> (3477)  
 <223> n equals a,t,g, or c

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&lt;210&gt; 75

&lt;211&gt; 928

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 75

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&lt;210&gt; 76

&lt;211&gt; 2636

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (632)

&lt;223&gt; n equals a,t,g, or c

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (887)

&lt;223&gt; n equals a,t,g, or c

&lt;400&gt; 76

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&lt;210&gt; 77

&lt;211&gt; 1320

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (831)

&lt;223&gt; n equals a,t,g, or c

&lt;400&gt; 77

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&lt;210&gt; 78

&lt;211&gt; 1259

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (4)

&lt;223&gt; n equals a,t,g, or c

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (18)

&lt;223&gt; n equals a,t,g, or c

&lt;400&gt; 78

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&lt;210&gt; 79

&lt;211&gt; 1420

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 79

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&lt;210&gt; 80

&lt;211&gt; 1183

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 80

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 <212> DNA  
 <213> Homo sapiens

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 <222> (6)  
 <223> n equals a,t,g, or c

<220>  
 <221> SITE  
 <222> (8)  
 <223> n equals a,t,g, or c

<220>  
 <221> SITE  
 <222> (48)  
 <223> n equals a,t,g, or c

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aaaaaaaaa	agggcgccg	c				1881

&lt;210&gt; 82

&lt;211&gt; 1433

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 82

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&lt;210&gt; 83

&lt;211&gt; 2454

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (2317)

&lt;223&gt; n equals a,t,g, or c

&lt;400&gt; 83

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&lt;210&gt; 84

&lt;211&gt; 1775

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (820)

&lt;223&gt; n equals a,t,g, or c



&lt;400&gt; 84

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&lt;210&gt; 85

&lt;211&gt; 1379

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 85

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63

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acaagcagtg tgaaaacacc gagggcggtt atcgtctcat ctgtgccgag ggctacaagc     1080
agatggaagg catctgtgtg aaggagcaga tcccagggtc attccccatc ttaactgatt     1140
taaccctga aacaaccga cgctggaagt tgggttctca tccccactct acatatgtaa     1200
aaatgaagat gcagagagat gaagctactt tcccagggtc atatggcaag caagtcgcaa     1260
agctgggatc ccaatccaga cagtctgacc gtggaacgag actcatacac gtaataaatg     1320
ctctgcccc cactgtcca ccacaaaaaa aaaaaaaaaa aaaaaaaaaa ggcgccgc      1379

```

<210> 86  
 <211> 700  
 <212> DNA  
 <213> Homo sapiens

```

<400> 86
ccacgcgtcc ggtccttgct tatgttttgg gagaccagc catctaccaa agcctgaagg      60
cacagaatgc gtattctcgt cactgtcctt tctatgtcag cattcagagt tactggctgt     120
catttttcat ggtgatgatt ttattttag ctttcataac ctgttgggaa gaagttacta     180
cttttggtaca ggctatcagg ataacttcct atatgaatga aactatctta tttttcctt     240
tttcatccca ctccagttat actgtgagat ctaaaaaat attcttatcc aagctcattg     300
tctgttttct cagtacctgg ttaccatttg tactacttca ggtaatcatt gttttactta     360
aagttcagat tccagcatat attgagatga atattccctg gttatacttt gtcaatagtt     420
ttctcattgc tacagtgtat tggtttaatt gtcacaagct taatttataa gacattggat     480
tacctttgga tccatttgtc aactggaagt gctgcttcat tccacttaca attcctaadc     540
ttgagcaaat tgaaaagcct atatcaataa tgatttggtta atattattaa ttaaaagtta     600
cagctgtcat aagatcataa ttttatgaac agaaagaact caggacatat taaaaataa     660
actgaactaa aacaaaaaaa aaaaaaaaaa aaaaaaaaaa

```

<210> 87  
 <211> 263  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (263)  
 <223> Xaa equals stop translation

```

<400> 87
Met Arg Leu Arg Leu Arg Leu Leu Ala Leu Leu Leu Leu Leu Ala
  1             5             10            15

Pro Pro Ala Arg Ala Pro Lys Pro Ser Ala Gln Asp Val Ser Leu Gly
      20             25            30

Val Asp Trp Leu Thr Arg Tyr Gly Tyr Leu Pro Pro Pro His Pro Ala

```

64

35	40	45
Gln Ala Gln Leu Gln Ser Pro Glu Lys Leu Arg Asp Ala Ile Lys Val		
50	55	60
Met Gln Arg Phe Ala Gly Leu Pro Glu Thr Gly Arg Met Asp Pro Gly		
65	70	75 80
Thr Val Ala Thr Met Arg Lys Pro Arg Cys Ser Leu Pro Asp Val Leu		
	85	90 95
Gly Val Ala Gly Leu Val Arg Arg Gly Arg Arg Tyr Ala Leu Ser Gly		
	100	105 110
Ser Val Trp Lys Lys Arg Thr Leu Thr Trp Arg Val Arg Ser Phe Pro		
	115	120 125
Gln Ser Ser Gln Leu Ser Gln Glu Thr Val Arg Val Leu Met Ser Tyr		
	130	135 140
Ala Leu Met Ala Trp Gly Met Glu Ser Gly Leu Thr Phe His Glu Val		
	145	150 155 160
Asp Ser Pro Gln Gly Gln Glu Pro Asp Ile Leu Ile Asp Phe Ala Arg		
	165	170 175
Ala Phe His Gln Asp Ser Tyr Pro Phe Asp Gly Leu Gly Gly Thr Leu		
	180	185 190
Ala His Ala Phe Phe Pro Gly Glu His Pro Ile Ser Gly Asp Thr His		
	195	200 205
Phe Asp Asp Glu Glu Thr Trp Thr Phe Gly Ser Lys Asp Gly Glu Gly		
	210	215 220
Thr Asp Leu Phe Ala Val Ala Val His Glu Phe Gly His Ala Leu Gly		
	225	230 235 240
Leu Gly His Ser Ser Ala Pro Asn Ser Ile Met Arg Pro Phe Tyr Gln		
	245	250 255
Gly Pro Val Gly Arg Pro Xaa		
	260	

&lt;210&gt; 88

&lt;211&gt; 206

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

65

&lt;221&gt; SITE

&lt;222&gt; (206)

&lt;223&gt; Xaa equals stop translation

&lt;400&gt; 88

Met Gly Thr Ala Gly Ala Met Gln Leu Cys Trp Val Ile Leu Gly Phe  
 1 5 10 15

Leu Leu Phe Arg Gly His Asn Ser Gln Pro Thr Met Thr Gln Thr Ser  
 20 25 30

Ser Ser Gln Gly Gly Leu Gly Gly Leu Ser Leu Thr Thr Glu Pro Val  
 35 40 45

Ser Ser Asn Pro Gly Tyr Ile Pro Ser Ser Glu Ala Asn Arg Pro Ser  
 50 55 60

His Leu Ser Ser Thr Gly Thr Pro Gly Ala Gly Val Pro Ser Ser Gly  
 65 70 75 80

Arg Asp Gly Gly Thr Ser Arg Asp Thr Phe Gln Thr Val Pro Pro Asn  
 85 90 95

Ser Thr Thr Met Ser Leu Ser Met Arg Glu Asp Ala Thr Ile Leu Pro  
 100 105 110

Ser Pro Thr Ser Glu Thr Val Leu Thr Val Ala Ala Phe Gly Val Ile  
 115 120 125

Ser Phe Ile Val Ile Leu Val Val Val Val Ile Ile Leu Val Gly Val  
 130 135 140

Val Ser Leu Arg Phe Lys Cys Arg Lys Ser Lys Glu Ser Glu Asp Pro  
 145 150 155 160

Gln Lys Pro Gly Ser Ser Gly Leu Ser Glu Ser Cys Ser Thr Ala Asn  
 165 170 175

Gly Glu Lys Asp Ser Ile Thr Leu Ile Ser Met Lys Asn Ile Asn Met  
 180 185 190

Asn Asn Gly Lys Gln Ser Leu Ser Ala Glu Lys Val Leu Xaa  
 195 200 205

&lt;210&gt; 89

&lt;211&gt; 673

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

66

&lt;221&gt; SITE

&lt;222&gt; (673)

&lt;223&gt; Xaa equals stop translation

&lt;400&gt; 89

Met Cys Ser Arg Val Pro Leu Leu Leu Pro Leu Leu Leu Leu Ala  
 1 5 10 15

Leu Gly Pro Gly Val Gln Gly Cys Pro Ser Gly Cys Gln Cys Ser Gln  
 20 25 30

Pro Gln Thr Val Phe Cys Thr Ala Arg Gln Gly Thr Thr Val Pro Arg  
 35 40 45

Asp Val Pro Pro Asp Thr Val Gly Leu Tyr Val Phe Glu Asn Gly Ile  
 50 55 60

Thr Met Leu Asp Ala Gly Ser Phe Ala Gly Leu Pro Gly Leu Gln Leu  
 65 70 75 80

Leu Asp Leu Ser Gln Asn Gln Ile Ala Ser Leu Pro Ser Gly Val Phe  
 85 90 95

Gln Pro Leu Ala Asn Leu Ser Asn Leu Asp Leu Thr Ala Asn Arg Leu  
 100 105 110

His Glu Ile Thr Asn Glu Thr Phe Arg Gly Leu Arg Arg Leu Glu Arg  
 115 120 125

Leu Tyr Leu Gly Lys Asn Arg Ile Arg His Ile Gln Pro Gly Ala Phe  
 130 135 140

Asp Thr Leu Asp Arg Leu Leu Glu Leu Lys Leu Gln Asp Asn Glu Leu  
 145 150 155 160

Arg Ala Leu Pro Pro Leu Arg Leu Pro Arg Leu Leu Leu Leu Asp Leu  
 165 170 175

Ser His Asn Ser Leu Leu Ala Leu Glu Pro Gly Ile Leu Asp Thr Ala  
 180 185 190

Asn Val Glu Ala Leu Arg Leu Ala Gly Leu Gly Leu Gln Gln Leu Asp  
 195 200 205

Glu Gly Leu Phe Ser Arg Leu Arg Asn Leu His Asp Leu Asp Val Ser  
 210 215 220

Asp Asn Gln Leu Glu Arg Val Pro Pro Val Ile Arg Gly Leu Arg Gly  
 225 230 235 240

Leu Thr Arg Leu Arg Leu Ala Gly Asn Thr Arg Ile Ala Gln Leu Arg

[illegible]

68

515                      520                      525  
 Ala Thr Tyr Ser Val Cys Val Met Pro Leu Gly Pro Gly Arg Val Pro  
 530                      535                      540  
 Glu Gly Glu Glu Ala Cys Gly Glu Ala His Thr Pro Pro Ala Val His  
 545                      550                      555                      560  
 Ser Asn His Ala Pro Val Thr Gln Ala Arg Glu Gly Asn Leu Pro Leu  
 565                      570                      575  
 Leu Ile Ala Pro Ala Leu Ala Ala Val Leu Leu Ala Ala Leu Ala Ala  
 580                      585                      590  
 Val Gly Ala Ala Tyr Cys Val Arg Arg Gly Arg Ala Met Ala Ala Ala  
 595                      600                      605  
 Ala Gln Asp Lys Gly Gln Val Gly Pro Gly Ala Gly Pro Leu Glu Leu  
 610                      615                      620  
 Glu Gly Val Lys Val Pro Leu Glu Pro Gly Pro Lys Ala Thr Glu Ala  
 625                      630                      635                      640  
 Val Glu Arg Pro Cys Pro Ala Gly Leu Ser Val Lys Cys His Ser Trp  
 645                      650                      655  
 Ala Ser Lys Ala Trp Pro Gln Ser Pro Leu His Ala Lys Pro Tyr Ile  
 660                      665                      670

Xaa

<210> 90  
 <211> 387  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (387)  
 <223> Xaa equals stop translation

<400> 90  
 Met Lys Phe Gln Gly Pro Leu Ala Cys Leu Leu Leu Ala Leu Cys Leu  
 1                      5                      10                      15  
 Gly Ser Gly Glu Ala Gly Pro Leu Gln Ser Gly Glu Glu Ser Thr Gly  
 20                      25                      30  
 Thr Asn Ile Gly Glu Ala Leu Gly His Gly Leu Gly Asp Ala Leu Ser

35	40	45
Glu Gly Val Gly Lys Ala Ile Gly Lys Glu Ala Gly Gly Ala Ala Gly		
50	55	60
Ser Lys Val Ser Glu Ala Leu Gly Gln Gly Thr Arg Glu Ala Val Gly		
65	70	75
Thr Gly Val Arg Gln Val Pro Gly Phe Gly Ala Ala Asp Ala Leu Gly		
85	90	95
Asn Arg Val Gly Glu Ala Ala His Ala Leu Gly Asn Thr Gly His Glu		
100	105	110
Ile Gly Arg Gln Ala Glu Asp Val Ile Arg His Gly Ala Asp Ala Val		
115	120	125
Arg Gly Ser Trp Gln Gly Val Pro Gly His Asn Gly Ala Trp Glu Thr		
130	135	140
Ser Gly Gly His Gly Ile Phe Gly Ser Gln Gly Gly Leu Gly Gly Gln		
145	150	155
Gly Gln Gly Asn Pro Gly Gly Leu Gly Thr Pro Trp Val His Gly Tyr		
165	170	175
Pro Gly Asn Ser Ala Gly Ser Phe Gly Met Asn Pro Gln Gly Ala Pro		
180	185	190
Trp Gly Gln Gly Gly Asn Gly Gly Pro Pro Asn Phe Gly Thr Asn Thr		
195	200	205
Gln Gly Ala Val Ala Gln Pro Gly Tyr Gly Ser Val Arg Ala Ser Asn		
210	215	220
Gln Asn Glu Gly Cys Thr Asn Pro Pro Pro Ser Gly Ser Gly Gly Gly		
225	230	235
Ser Ser Asn Ser Gly Gly Gly Ser Gly Ser Gln Ser Gly Ser Ser Gly		
245	250	255
Ser Gly Ser Asn Gly Asp Asn Asn Asn Gly Ser Ser Ser Gly Gly Ser		
260	265	270
Ser Ser Gly Ser Ser Ser Gly Gly Ser Ser Gly Gly Ser Ser Gly Gly		
275	280	285
Ser Ser Gly Asn Ser Gly Gly Ser Arg Gly Asp Ser Gly Ser Glu Ser		
290	295	300
Ser Trp Gly Ser Ser Thr Gly Ser Ser Ser Gly Asn His Gly Gly Ser		



70

305                                      310                                      315                                      320  
 Gly Gly Gly Asn Gly His Lys Pro Gly Cys Glu Lys Pro Gly Asn Glu  
    325                                      330                                      335  
 Ala Arg Gly Ser Gly Glu Ser Gly Ile Gln Asn Ser Glu Thr Ser Pro  
    340                                      345                                      350  
 Gly Met Phe Asn Phe Asp Thr Phe Trp Lys Asn Phe Lys Ser Lys Leu  
    355                                      360                                      365  
 Gly Phe Ile Asn Trp Asp Ala Ile Asn Lys Asp Gln Arg Ser Ser Arg  
    370                                      375                                      380  
 Ile Pro Xaa  
 385

<210> 91  
 <211> 96  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (96)  
 <223> Xaa equals stop translation

<400> 91  
 Met Thr Leu Ala Leu Ala Tyr Leu Leu Ala Leu Pro Gln Val Leu Asp  
   1  5  10  15  
 Ala Asn Arg Cys Phe Glu Lys Gln Ser Pro Ser Ala Leu Ser Leu Gln  
   20  25  30  
 Leu Ala Ala Tyr Tyr Tyr Ser Leu Gln Ile Tyr Ala Arg Leu Ala Pro  
   35  40  45  
 Cys Phe Arg Asp Lys Cys His Pro Leu Tyr Arg Glu Leu Ile Thr Tyr  
   50  55  60  
 Val Ser Arg Met Tyr Ser Lys Trp Gln Ala Ala Leu Gly Phe Pro Val  
   65  70  75  80  
 Phe Asp Lys Val Ala Ser Pro Gly Ile Ser Trp Arg Thr Val Val Xaa  
   85  90  95

<210> 92  
<211> 121  
<212> PRT  
<213> Homo sapiens

<220>  
<221> SITE  
<222> (121)  
<223> Xaa equals stop translation

<400> 92  
Met Leu Asn Leu Gly Ser Trp Pro Gly Leu Val Ala Ala Ser Leu Phe  
1 5 10 15  
Leu Leu Lys Gly Val Phe Ser Leu Phe Val Gln Leu Leu Lys Asn Pro  
20 25 30  
Leu Gln His Pro Arg Asn Arg Ala Thr His Leu Leu Ala Thr Pro Gly  
35 40 45  
Ala Arg Val Leu Gln Glu His Leu Ser Ile His Pro Val Cys His Gln  
50 55 60  
Ser His Pro Pro Glu Ala Pro Leu Leu Pro Pro Ser Thr Arg Ala Ser  
65 70 75 80  
Leu Gln Ala Ser Pro Pro Pro Pro Ser Ser Gln His Pro Gly Gly  
85 90 95  
Thr Pro Ala Ala Cys Leu Gln Ser Lys Leu Pro Ile Thr His Arg Arg  
100 105 110  
Ser Pro Leu Arg Arg Pro Arg His Xaa  
115 120

<210> 93  
<211> 122  
<212> PRT  
<213> Homo sapiens

<220>  
<221> SITE  
<222> (122)  
<223> Xaa equals stop translation

<400> 93  
Met Cys Phe Leu Met Ile Phe Thr Phe Leu Val Cys Trp Met Pro Tyr  
1 5 10 15  
Ile Val Ile Cys Phe Leu Val Val Asn Gly His Gly His Leu Val Thr

20										25					30				
Pro	Thr	Ile	Ser	Ile	Val	Ser	Tyr	Leu	Phe	Ala	Lys	Ser	Asn	Thr	Val				
35								40				45							
Tyr	Asn	Pro	Val	Ile	Tyr	Val	Phe	Met	Ile	Arg	Lys	Phe	Arg	Arg	Ser				
50					55				60										
Leu	Leu	Gln	Leu	Leu	Cys	Leu	Arg	Leu	Leu	Arg	Cys	Gln	Arg	Pro	Ala				
65		70					75					80							
Lys	Asp	Leu	Pro	Ala	Ala	Gly	Ser	Glu	Met	Gln	Ile	Arg	Pro	Ile	Val				
85						90					95								
Met	Ser	Gln	Lys	Asp	Gly	Asp	Arg	Pro	Lys	Lys	Ser	Asp	Phe	Gln	Leu				
100				105				110											
Phe	Phe	His	His	Phe	Tyr	His	His	Gln	Xaa										
115				120															

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<210> 94
<211> 102
<212> PRT
<213> Homo sapiens
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<220>  
<221> SITE  
<222> (102)  
<223> Xaa equals stop translation
```

```

<400> 94
Met Lys Gln Arg Leu Arg Gly Gln Gln Gly Phe Gln Leu Asp Val Cys
 1             5             10             15

Val Ala Cys Thr Leu Leu Phe Leu Leu Leu Thr Val Asn Ser Gly Val
      20             25             30

Thr Ser Arg Glu Gln Leu Gly Cys Ser Arg Pro Ser Pro Ala Gln Gly
      35             40             45

Glu Gly Arg Gly Thr Cys Ser Ser Glu Gln Pro Glu Gly Gly Gly Arg
 50             55             60

Ser Glu Val Val Glu Trp Phe Val Tyr Leu Thr Gly Leu Lys Gly Pro
 65             70             75             80

Ser Val Phe Val Val Cys Phe Val Ser Cys Phe Ser Asp Arg Ser Ile
      85             90             95

Thr Thr Asp Leu Leu Xaa

```

100

<210> 95  
 <211> 186  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (186)  
 <223> Xaa equals stop translation

<400> 95  
 Met Lys Phe Thr Ile Val Phe Ala Gly Leu Leu Gly Val Phe Leu Ala  
     1                    5                    10                    15  
 Pro Ala Leu Ala Asn Tyr Asn Ile Asn Val Asn Asp Asp Asn Asn Asn  
                     20                    25                    30  
 Ala Gly Ser Gly Gln Gln Ser Val Ser Val Asn Asn Glu His Asn Val  
                     35                    40                    45  
 Ala Asn Val Asp Asn Asn Asn Gly Trp Asp Ser Trp Asn Ser Ile Trp  
                     50                    55                    60  
 Asp Tyr Gly Asn Gly Phe Ala Ala Thr Arg Leu Phe Gln Lys Lys Thr  
     65                    70                    75                    80  
 Cys Ile Val His Lys Met Asn Lys Glu Val Met Pro Ser Ile Gln Ser  
                     85                    90                    95  
 Leu Asp Ala Leu Val Lys Glu Lys Lys Leu Gln Gly Lys Gly Pro Gly  
                     100                    105                    110  
 Gly Pro Pro Pro Lys Gly Leu Met Tyr Ser Val Asn Pro Asn Lys Val  
                     115                    120                    125  
 Asp Asp Leu Ser Lys Phe Gly Lys Asn Ile Ala Asn Met Cys Arg Gly  
     130                    135                    140  
 Ile Pro Thr Tyr Met Ala Glu Glu Met Gln Glu Ala Ser Leu Phe Phe  
     145                    150                    155                    160  
 Tyr Ser Gly Thr Cys Tyr Thr Thr Ser Val Leu Trp Ile Val Asp Ile  
                     165                    170                    175  
 Ser Phe Cys Gly Asp Thr Val Glu Asn Xaa  
                     180                    185

<210> 96  
 <211> 232  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (232)  
 <223> Xaa equals stop translation

<400> 96

Met Ser Arg Ala Met Ala Leu Phe Phe Val Leu Cys Trp Ile Gln Gly  
 1 5 10 15

Tyr Ser Gln Gln Lys Ser Leu Asn Asn Ala Ala Phe Ala Ser Gly Ser  
 20 25 30

Asn Glu Arg Glu Glu His Leu Ala Lys Ile Phe Asp Glu Ile Leu Leu  
 35 40 45

Gln Val Phe Pro Lys Phe Pro Tyr Asp Pro Ser Phe Asn Glu Ala Thr  
 50 55 60

Ala Val Arg Ser Ile Thr Lys Thr Asp Met Arg Lys Gly Thr Ser Ile  
 65 70 75 80

Ala Trp Asn Ser Pro Lys Pro Glu Tyr Phe Leu Gly Ser Val Asp Lys  
 85 90 95

Ile Pro Asp Lys Asp His Leu Ser Glu Glu Lys Asn Phe Lys Glu Ser  
 100 105 110

Cys Leu Phe Asp Arg Asp Leu Arg Glu Gln Leu Thr Thr Ile Asp Lys  
 115 120 125

Glu Thr Leu Gln Gly Ala Ala Lys Pro Asp Ala His Phe Arg Thr Met  
 130 135 140

Pro Cys Gly Gln Leu Leu His Phe Leu Gln Arg Asn Thr Ile Ile Ala  
 145 150 155 160

Thr Val Ser Gly Val Ala Ile Leu Met Ala Ile Val Leu Leu Leu Leu  
 165 170 175

Gly Leu Ala Ser Tyr Ile Arg Lys Lys Gln Pro Ser Ser Pro Leu Ala  
 180 185 190

Asn Thr Thr Tyr Asn Ile Phe Ile Met Asp Gly Lys Thr Trp Trp His  
 195 200 205

Asn Ser Glu Glu Lys Asn Phe Thr Lys Leu Ala Lys Lys Gln Lys Gln

75

210 215 220

Leu Lys Ser Ser Ser Cys Val Xaa  
225 230

<210> 97  
<211> 137  
<212> PRT  
<213> Homo sapiens

<220>  
<221> SITE  
<222> (137)  
<223> Xaa equals stop translation

<400> 97  
Met Ala Ser Leu Gly Leu Leu Leu Leu Leu Leu Thr Ala Leu Pro  
1 5 10 15  
Pro Leu Trp Ser Ser Ser Leu Pro Gly Leu Asp Thr Ala Glu Ser Lys  
20 25 30  
Ala Thr Ile Ala Asp Leu Ile Leu Ser Ala Leu Glu Arg Ala Thr Val  
35 40 45  
Phe Leu Glu Gln Arg Leu Pro Glu Ile Asn Leu Asp Gly Met Val Gly  
50 55 60  
Val Arg Val Leu Glu Glu Gln Leu Lys Ser Val Arg Glu Lys Trp Ala  
65 70 75 80  
Gln Glu Pro Leu Leu Gln Pro Leu Ser Leu Arg Val Gly Met Leu Gly  
85 90 95  
Glu Lys Leu Glu Ala Ala Ile Gln Arg Ser Leu His Tyr Leu Lys Leu  
100 105 110  
Ser Asp Pro Lys Tyr Leu Arg Gly Arg Thr Ala Ala Ser Pro Ala Ala  
115 120 125  
Ser Gln Thr Ser Ala Gly Ala Ser Xaa  
130 135

<210> 98  
<211> 607  
<212> PRT  
<213> Homo sapiens

<220>

&lt;221&gt; SITE

&lt;222&gt; (607)

&lt;223&gt; Xaa equals stop translation

&lt;400&gt; 98

Met Thr Val Val Gly Asn Pro Arg Ser Trp Ser Cys Gln Trp Leu Pro  
 1 5 10 15

Ile Leu Ile Leu Leu Leu Gly Thr Gly His Gly Pro Gly Val Glu Gly  
 20 25 30

Val Thr His Tyr Lys Ala Gly Asp Pro Val Ile Leu Tyr Val Asn Lys  
 35 40 45

Val Gly Pro Tyr His Asn Pro Gln Glu Thr Tyr His Tyr Tyr Gln Leu  
 50 55 60

Pro Val Cys Cys Pro Glu Lys Ile Arg His Lys Ser Leu Ser Leu Gly  
 65 70 75 80

Glu Val Leu Asp Gly Asp Arg Met Ala Glu Ser Leu Tyr Glu Ile Arg  
 85 90 95

Phe Arg Glu Asn Val Glu Lys Arg Ile Leu Cys His Met Gln Leu Ser  
 100 105 110

Ser Ala Gln Val Glu Gln Leu Arg Gln Ala Ile Glu Glu Leu Tyr Tyr  
 115 120 125

Phe Glu Phe Val Val Asp Asp Leu Pro Ile Arg Gly Phe Val Gly Tyr  
 130 135 140

Met Glu Glu Ser Gly Phe Leu Pro His Ser His Lys Ile Gly Leu Trp  
 145 150 155 160

Thr His Leu Asp Phe His Leu Glu Phe His Gly Asp Arg Ile Ile Phe  
 165 170 175

Ala Asn Val Ser Val Arg Asp Val Lys Pro His Ser Leu Asp Gly Leu  
 180 185 190

Arg Pro Asp Glu Phe Leu Gly Leu Thr His Thr Tyr Ser Val Arg Trp  
 195 200 205

Ser Glu Thr Ser Val Glu Arg Arg Ser Asp Arg Arg Arg Gly Asp Asp  
 210 215 220

Gly Gly Phe Phe Pro Arg Thr Leu Glu Ile His Trp Leu Ser Ile Ile  
 225 230 235 240

Asn Ser Met Val Leu Val Phe Leu Leu Val Gly Phe Val Ala Val Ile

Leu	Met	Arg	Val	Leu	Arg	Asn	Asp	Leu	Ala	Arg	Tyr	Asn	Leu	Asp	Glu	
			260					265					270			
Glu	Thr	Thr	Ser	Ala	Gly	Ser	Gly	Asp	Asp	Phe	Asp	Gln	Gly	Asp	Asn	
		275					280					285				
Gly	Trp	Lys	Ile	Ile	His	Thr	Asp	Val	Phe	Arg	Phe	Pro	Pro	Tyr	Arg	
	290					295					300					
Gly	Leu	Leu	Cys	Ala	Val	Leu	Gly	Val	Gly	Ala	Gln	Phe	Leu	Ala	Leu	
305					310					315					320	
Gly	Thr	Gly	Ile	Ile	Val	Met	Ala	Leu	Leu	Gly	Met	Phe	Asn	Val	His	
				325					330					335		
Arg	His	Gly	Ala	Ile	Asn	Ser	Ala	Ala	Ile	Leu	Leu	Tyr	Ala	Leu	Thr	
			340					345					350			
Cys	Cys	Ile	Ser	Gly	Tyr	Val	Ser	Ser	His	Phe	Tyr	Arg	Gln	Ile	Gly	
		355					360					365				
Gly	Glu	Arg	Trp	Val	Trp	Asn	Ile	Ile	Leu	Thr	Thr	Ser	Leu	Phe	Ser	
	370					375						380				
Val	Pro	Phe	Phe	Leu	Thr	Trp	Ser	Val	Val	Asn	Ser	Val	His	Trp	Ala	
385					390					395					400	
Asn	Gly	Ser	Thr	Gln	Ala	Leu	Pro	Ala	Thr	Thr	Ile	Leu	Leu	Leu	Leu	
				405					410					415		
Thr	Val	Trp	Leu	Leu	Val	Gly	Phe	Pro	Leu	Thr	Val	Ile	Gly	Gly	Ile	
			420					425					430			
Phe	Gly	Lys	Asn	Asn	Ala	Ser	Pro	Phe	Asp	Ala	Pro	Cys	Arg	Thr	Lys	
		435					440					445				
Asn	Ile	Ala	Arg	Glu	Ile	Pro	Pro	Gln	Pro	Trp	Tyr	Lys	Ser	Thr	Val	
	450					455					460					
Ile	His	Met	Thr	Val	Gly	Gly	Phe	Leu	Pro	Phe	Ser	Ala	Ile	Ser	Val	
465					470					475					480	
Glu	Leu	Tyr	Tyr	Ile	Phe	Ala	Thr	Val	Trp	Gly	Arg	Glu	Gln	Tyr	Thr	
				485					490					495		
Leu	Tyr	Gly	Ile	Leu	Phe	Phe	Val	Phe	Ala	Ile	Leu	Leu	Ser	Val	Gly	
			500					505					510			
Ala	Cys	Ile	Ser	Ile	Ala	Leu	Thr	Tyr	Phe	Gln	Leu	Ser	Gly	Glu	Asp	



515                      520                      525  
 Tyr Arg Trp Trp Trp Arg Ser Val Leu Ser Val Gly Ser Thr Gly Leu  
 530                      535                      540  
 Phe Ile Phe Leu Tyr Ser Val Phe Tyr Tyr Ala Arg Arg Ser Asn Met  
 545                      550                      555                      560  
 Ser Gly Ala Val Gln Thr Val Glu Phe Phe Gly Tyr Ser Leu Leu Thr  
 565                      570                      575  
 Gly Tyr Val Phe Phe Leu Met Leu Gly Thr Ile Ser Phe Phe Ser Ser  
 580                      585                      590  
 Leu Lys Phe Ile Arg Tyr Ile Tyr Val Asn Leu Lys Met Asp Xaa  
 595                      600                      605

&lt;210&gt; 99

&lt;211&gt; 311

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (311)

&lt;223&gt; Xaa equals stop translation

&lt;400&gt; 99

Met Ala Leu Arg Arg Pro Pro Arg Leu Arg Leu Cys Ala Arg Leu Pro  
 1                      5                      10                      15

Asp Phe Phe Leu Leu Leu Leu Phe Arg Gly Cys Leu Ile Gly Ala Val  
 20                      25                      30

Asn Leu Lys Ser Ser Asn Arg Thr Pro Val Val Gln Glu Phe Glu Ser  
 35                      40                      45

Val Glu Leu Ser Cys Ile Ile Thr Asp Ser Gln Thr Ser Asp Pro Arg  
 50                      55                      60

Ile Glu Trp Lys Lys Ile Gln Asp Glu Gln Thr Thr Tyr Val Phe Phe  
 65                      70                      75                      80

Asp Asn Lys Ile Gln Gly Asp Leu Ala Gly Arg Ala Glu Ile Leu Gly  
 85                      90                      95

Lys Thr Ser Leu Lys Ile Trp Asn Val Thr Arg Arg Asp Ser Ala Leu  
 100                      105                      110

Tyr Arg Cys Glu Val Val Ala Arg Asn Asp Arg Lys Glu Ile Asp Glu

115	120	125
Ile Val Ile Glu Leu Thr Val Gln Val Lys Pro Val Thr Pro Val Cys		
130	135	140
Arg Val Pro Lys Ala Val Pro Val Gly Lys Met Ala Thr Leu His Cys		
145	150	155
Gln Glu Ser Glu Gly His Pro Arg Pro His Tyr Ser Trp Tyr Arg Asn		
165	170	175
Asp Val Pro Leu Pro Thr Asp Ser Arg Ala Asn Pro Arg Phe Arg Asn		
180	185	190
Ser Ser Phe His Leu Asn Ser Glu Thr Gly Thr Leu Val Phe Thr Ala		
195	200	205
Val His Lys Asp Asp Ser Gly Gln Tyr Tyr Cys Ile Ala Ser Asn Asp		
210	215	220
Ala Gly Ser Ala Arg Cys Glu Glu Gln Glu Met Glu Val Tyr Asp Leu		
225	230	235
Asn Ile Gly Gly Ile Ile Gly Gly Val Leu Val Val Leu Ala Val Leu		
245	250	255
Ala Leu Ile Thr Leu Gly Ile Cys Cys Ala Tyr Arg Arg Gly Tyr Phe		
260	265	270
Ile Asn Asn Lys Gln Asp Gly Glu Ser Tyr Lys Asn Pro Gly Lys Pro		
275	280	285
Asp Gly Val Asn Tyr Ile Arg Thr Asp Glu Glu Gly Asp Phe Arg His		
290	295	300
Lys Ser Ser Phe Val Ile Xaa		
305	310	

&lt;210&gt; 100

&lt;211&gt; 247

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 100

Met Glu Lys Cys Leu Gln Asp Phe Cys Leu Pro Phe Leu Arg Ile Thr
1 5 10 15

Ser Leu Leu Gln His His Leu Phe Gly Glu Asp Leu Pro Ser Cys Gln
20 25 30

80

Glu Glu Glu Glu Phe Ser Val Leu Ala Ser Cys Leu Gly Leu Leu Pro  
 35 40 45  
 Thr Phe Tyr Gln Thr Glu His Pro Phe Ile Ser Ala Ser Cys Leu Asp  
 50 55 60  
 Trp Pro Val Pro Ala Phe Asp Ile Ile Thr Gln Trp Cys Phe Glu Ile  
 65 70 75 80  
 Lys Ser Phe Thr Glu Arg His Ala Glu Gln Gly Lys Ala Leu Leu Ile  
 85 90 95  
 Gln Glu Ser Lys Trp Lys Leu Pro His Leu Leu Gln Leu Pro Glu Asn  
 100 105 110  
 Tyr Asn Thr Ile Phe Gln Tyr Tyr His Arg Lys Thr Cys Ser Val Cys  
 115 120 125  
 Thr Lys Val Pro Lys Asp Pro Ala Val Cys Leu Val Cys Gly Thr Phe  
 130 135 140  
 Val Cys Leu Lys Gly Leu Cys Cys Lys Gln Gln Ser Tyr Cys Glu Cys  
 145 150 155 160  
 Val Leu His Ser Gln Asn Cys Gly Ala Gly Thr Gly Ile Phe Leu Leu  
 165 170 175  
 Ile Asn Ala Ser Val Ile Ile Ile Ile Arg Gly His Arg Phe Cys Leu  
 180 185 190  
 Trp Gly Ser Val Tyr Leu Asp Ala His Gly Glu Glu Asp Arg Asp Leu  
 195 200 205  
 Arg Arg Gly Lys Pro Leu Tyr Ile Cys Lys Glu Arg Tyr Lys Val Leu  
 210 215 220  
 Glu Gln Gln Trp Ile Ser His Thr Phe Asp His Ile Asn Lys Arg Trp  
 225 230 235 240  
 Gly Pro His Tyr Asn Gly Leu  
 245

&lt;210&gt; 101

&lt;211&gt; 560

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (560)

<223> Xaa equals stop translation

<400> 101

Met	Val	Leu	Leu	His	Trp	Cys	Leu	Leu	Trp	Leu	Leu	Phe	Pro	Leu	Ser
1				5					10					15	
Ser	Arg	Thr	Gln	Lys	Leu	Pro	Thr	Arg	Asp	Glu	Glu	Leu	Phe	Gln	Met
			20					25					30		
Gln	Ile	Arg	Asp	Lys	Ala	Phe	Phe	His	Asp	Ser	Ser	Val	Ile	Pro	Asp
		35					40					45			
Gly	Ala	Glu	Ile	Ser	Ser	Tyr	Leu	Phe	Arg	Asp	Thr	Pro	Lys	Arg	Tyr
	50					55					60				
Phe	Phe	Val	Val	Glu	Glu	Asp	Asn	Thr	Pro	Leu	Ser	Val	Thr	Val	Thr
65					70					75					80
Pro	Cys	Asp	Ala	Pro	Leu	Glu	Trp	Lys	Leu	Ser	Leu	Gln	Glu	Leu	Pro
			85						90					95	
Glu	Asp	Arg	Ser	Gly	Glu	Gly	Ser	Gly	Asp	Leu	Glu	Pro	Leu	Glu	Gln
			100					105					110		
Gln	Lys	Gln	Gln	Ile	Ile	Asn	Glu	Glu	Gly	Thr	Glu	Leu	Phe	Ser	Tyr
		115					120					125			
Lys	Gly	Asn	Asp	Val	Glu	Tyr	Phe	Ile	Ser	Ser	Ser	Ser	Pro	Ser	Gly
	130					135					140				
Leu	Tyr	Gln	Leu	Asp	Leu	Leu	Ser	Thr	Glu	Lys	Asp	Thr	His	Phe	Lys
145				150						155					160
Val	Tyr	Ala	Thr	Thr	Thr	Pro	Glu	Ser	Asp	Gln	Pro	Tyr	Pro	Glu	Leu
				165					170					175	
Pro	Tyr	Asp	Pro	Arg	Val	Asp	Val	Thr	Ser	Leu	Gly	Arg	Thr	Thr	Val
			180					185					190		
Thr	Leu	Ala	Trp	Lys	Pro	Ser	Pro	Thr	Ala	Ser	Leu	Leu	Lys	Gln	Pro
		195					200					205			
Ile	Gln	Tyr	Cys	Val	Val	Ile	Asn	Lys	Glu	His	Asn	Phe	Lys	Ser	Leu
	210					215					220				
Cys	Ala	Val	Glu	Ala	Lys	Leu	Ser	Ala	Asp	Asp	Ala	Phe	Met	Met	Ala
225					230					235					240
Pro	Lys	Pro	Gly	Leu	Asp	Phe	Ser	Pro	Phe	Asp	Phe	Ala	His	Phe	Gly
				245					250					255	

Phe Pro Ser Asp Asn Ser Gly Lys Glu Arg Ser Phe Gln Ala Lys Pro  
 260 265 270  
 Ser Pro Lys Leu Gly Arg His Val Tyr Ser Arg Pro Lys Val Asp Ile  
 275 280 285  
 Gln Lys Ile Cys Ile Gly Asn Lys Asn Ile Phe Thr Val Ser Asp Leu  
 290 295 300  
 Lys Pro Asp Thr Gln Tyr Tyr Phe Asp Val Phe Val Val Asn Ile Asn  
 305 310 315 320  
 Ser Asn Met Ser Thr Ala Tyr Val Gly Thr Phe Ala Arg Thr Lys Glu  
 325 330 335  
 Glu Ala Lys Gln Lys Thr Val Glu Leu Lys Asp Gly Lys Ile Thr Asp  
 340 345 350  
 Val Phe Val Lys Arg Lys Gly Ala Lys Phe Leu Arg Phe Ala Pro Val  
 355 360 365  
 Ser Ser His Gln Lys Val Thr Phe Phe Ile His Ser Cys Leu Asp Ala  
 370 375 380  
 Val Gln Ile Gln Val Arg Arg Asp Gly Lys Leu Leu Leu Ser Gln Asn  
 385 390 395 400  
 Val Glu Gly Ile Gln Gln Phe Gln Leu Arg Gly Lys Pro Lys Ala Lys  
 405 410 415  
 Tyr Leu Val Arg Leu Lys Gly Asn Lys Lys Gly Ala Ser Met Leu Lys  
 420 425 430  
 Ile Leu Ala Thr Thr Arg Pro Thr Lys Gln Ser Phe Pro Ser Leu Pro  
 435 440 445  
 Glu Asp Thr Arg Ile Lys Ala Phe Asp Lys Leu Arg Thr Cys Ser Ser  
 450 455 460  
 Ala Thr Val Ala Trp Leu Gly Thr Gln Glu Arg Asn Lys Phe Cys Ile  
 465 470 475 480  
 Tyr Lys Lys Glu Val Asp Asp Asn Tyr Asn Glu Asp Gln Lys Lys Arg  
 485 490 495  
 Glu Gln Asn Gln Cys Leu Gly Pro Asp Ile Arg Lys Lys Ser Glu Lys  
 500 505 510  
 Val Leu Cys Lys Tyr Phe His Ser Gln Asn Leu Gln Lys Ala Val Thr  
 515 520 525

Thr Glu Thr Ile Lys Gly Leu Gln Pro Gly Lys Ser Leu Pro Ala Gly  
 530 535 540

Cys Leu Cys His Arg Thr Trp Gly Ala Leu Cys Lys Val Ser Glu Xaa  
 545 550 555 560

<210> 102

<211> 72

<212> PRT

<213> Homo sapiens

<220>

<221> SITE

<222> (72)

<223> Xaa equals stop translation

<400> 102

Met Ser Pro Ser His Ser Pro Val Ser Cys Phe Lys Leu Arg Val Leu  
 1 5 10 15

Val Phe Pro Leu Pro Leu Phe Leu Gly Thr Ala Leu Cys Ser Val Trp  
 20 25 30

Asp Pro Arg Ala Arg Pro Leu Gly Leu Val Ala Ala Ala Arg Pro Leu  
 35 40 45

Gly Pro Ser Thr Cys Pro Ser Pro Arg Phe Pro Ala Ser Ser Ala Gly  
 50 55 60

Thr Leu Lys Leu Arg Ala Arg Xaa  
 65 70

<210> 103

<211> 159

<212> PRT

<213> Homo sapiens

<220>

<221> SITE

<222> (159)

<223> Xaa equals stop translation

<400> 103

Met Ala Leu Glu Val Leu Met Leu Leu Ala Val Leu Ile Trp Thr Gly  
 1 5 10 15

84

Ala Glu Asn Leu His Val Lys Ile Ser Cys Ser Leu Asp Trp Leu Met  
 20 25 30

Val Ser Val Ile Pro Val Ala Glu Ser Arg Asn Leu Tyr Ile Phe Ala  
 35 40 45

Asp Glu Leu His Leu Gly Met Gly Cys Pro Ala Asn Arg Ile His Thr  
 50 55 60

Tyr Val Tyr Glu Phe Ile Tyr Leu Val Arg Asp Cys Gly Ile Arg Thr  
 65 70 75 80

Arg Val Val Ser Glu Glu Thr Leu Leu Phe Gln Thr Glu Leu Tyr Phe  
 85 90 95

Thr Pro Arg Asn Ile Asp His Asp Pro Gln Glu Ile His Leu Glu Cys  
 100 105 110

Ser Thr Ser Arg Lys Ser Val Trp Leu Thr Pro Val Ser Thr Glu Asn  
 115 120 125

Glu Ile Lys Leu Asp Pro Ser Pro Phe Ile Ala Asp Phe Gln Thr Thr  
 130 135 140

Ala Glu Glu Leu Gly Leu Leu Ser Ser Ser Pro Asn Leu Leu Xaa  
 145 150 155

<210> 104  
 <211> 170  
 <212> PRT  
 <213> Homo sapiens

<400> 104  
 Met Ile Leu Thr Met Leu Leu Met Leu Lys Leu Cys Thr Glu Val Arg  
 1 5 10 15

Val Ala Asn Glu Leu Asn Ala Arg Arg Arg Ser Phe Thr Asp Phe Asp  
 20 25 30

Pro His His Phe Trp Gln Trp Ser Ser Phe Ser Asp Tyr Val Gln Cys  
 35 40 45

Val Leu Ala Phe Thr Gly Val Ala Gly Tyr Ile Thr Tyr Leu Ser Ile  
 50 55 60

Asp Ser Ala Leu Phe Val Glu Thr Leu Gly Phe Leu Ala Val Leu Thr  
 65 70 75 80

Glu Ala Met Leu Gly Val Pro Gln Leu Tyr Arg Asn His Arg His Gln  
 85 90 95

85

Ser Thr Glu Gly Met Ser Ile Lys Met Val Leu Met Trp Thr Ser Gly  
 100 105 110

Asp Ala Phe Lys Thr Ala Tyr Phe Leu Leu Lys Gly Ala Pro Leu Gln  
 115 120 125

Phe Ser Val Cys Gly Leu Leu Gln Val Leu Val Asp Leu Ala Ile Leu  
 130 135 140

Gly Gln Ala Tyr Ala Phe Ala Arg His Pro Gln Lys Pro Ala Pro His  
 145 150 155 160

Ala Val His Pro Thr Gly Thr Lys Ala Leu  
 165 170

&lt;210&gt; 105

&lt;211&gt; 355

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (10)

&lt;223&gt; Xaa equals any of the naturally occurring L-amino acids

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (355)

&lt;223&gt; Xaa equals stop translation

&lt;400&gt; 105

Met Ala Gly Pro Arg Leu Leu Phe Leu Xaa Ala Leu Ala Leu Glu Leu  
 1 5 10 15

Leu Gly Arg Ala Gly Gly Ser Gln Pro Ala Leu Arg Ser Arg Gly Thr  
 20 25 30

Ala Thr Ala Cys Arg Leu Asp Asn Lys Glu Ser Glu Ser Trp Gly Ala  
 35 40 45

Leu Leu Ser Gly Glu Arg Leu Asp Thr Trp Ile Cys Ser Leu Leu Gly  
 50 55 60

Ser Leu Met Val Gly Leu Ser Gly Val Phe Pro Leu Leu Val Ile Pro  
 65 70 75 80

Leu Glu Met Gly Thr Met Leu Arg Ser Glu Ala Gly Ala Trp Arg Leu  
 85 90 95



Lys Gln Leu Leu Ser Phe Ala Leu Gly Gly Leu Leu Gly Asn Val Phe  
 100 105 110  
 Leu His Leu Leu Pro Glu Ala Trp Ala Tyr Thr Cys Ser Ala Ser Pro  
 115 120 125  
 Gly Gly Glu Gly Gln Ser Leu Gln Gln Gln Gln Leu Gly Leu Trp  
 130 135 140  
 Val Ile Ala Gly Ile Leu Thr Phe Leu Ala Leu Glu Lys Met Phe Leu  
 145 150 155 160  
 Asp Ser Lys Glu Glu Gly Thr Ser Gln Ala Pro Asn Lys Asp Pro Thr  
 165 170 175  
 Ala Ala Ala Ala Ala Leu Asn Gly Gly His Cys Leu Ala Gln Pro Ala  
 180 185 190  
 Ala Glu Pro Gly Leu Gly Ala Val Val Arg Ser Ile Lys Val Ser Gly  
 195 200 205  
 Tyr Leu Asn Leu Leu Ala Asn Thr Ile Asp Asn Phe Thr His Gly Leu  
 210 215 220  
 Ala Val Ala Ala Ser Phe Leu Val Ser Lys Lys Ile Gly Leu Leu Thr  
 225 230 235 240  
 Thr Met Ala Ile Leu Leu His Glu Ile Pro His Glu Val Gly Asp Phe  
 245 250 255  
 Ala Ile Leu Leu Arg Ala Gly Phe Asp Arg Trp Ser Ala Ala Lys Leu  
 260 265 270  
 Gln Leu Ser Thr Ala Leu Gly Gly Leu Leu Gly Ala Gly Phe Ala Ile  
 275 280 285  
 Cys Thr Gln Ser Pro Lys Gly Val Glu Glu Thr Ala Ala Trp Val Leu  
 290 295 300  
 Pro Phe Thr Ser Gly Gly Phe Leu Tyr Ile Ala Leu Val Asn Val Leu  
 305 310 315 320  
 Pro Asp Leu Leu Glu Glu Glu Asp Pro Trp Arg Ser Leu Gln Gln Leu  
 325 330 335  
 Leu Leu Leu Cys Ala Gly Ile Val Val Met Val Leu Phe Ser Leu Phe  
 340 345 350  
 Val Asp Xaa  
 355

<210> 106  
 <211> 146  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (146)  
 <223> Xaa equals stop translation

<400> 106  
 Met Ser Gln Ala Trp Val Pro Gly Leu Ala Pro Thr Leu Leu Phe Ser  
       1                  5                  10                  15  
 Leu Leu Ala Gly Pro Gln Lys Ile Ala Ala Lys Cys Gly Leu Ile Leu  
                   20                  25                  30  
 Ala Cys Pro Lys Gly Phe Lys Cys Cys Gly Asp Ser Cys Cys Gln Glu  
                   35                  40                  45  
 Asn Glu Leu Phe Pro Gly Pro Val Arg Ile Phe Val Ile Ile Phe Leu  
       50                  55                  60  
 Val Ile Leu Ser Val Phe Cys Ile Cys Gly Leu Ala Lys Cys Phe Cys  
       65                  70                  75                  80  
 Arg Asn Cys Arg Glu Pro Glu Pro Asp Ser Pro Val Asp Cys Arg Gly  
                   85                  90                  95  
 Pro Leu Glu Leu Pro Ser Ile Ile Pro Pro Glu Arg Val Ile Leu Lys  
                   100                  105                  110  
 Pro Ser Leu Gly Pro Thr Pro Thr Glu Pro Pro Pro Pro Tyr Ser Phe  
                   115                  120                  125  
 Arg Pro Glu Glu Tyr Thr Gly Asp Gln Arg Gly Ile Asp Asn Pro Ala  
       130                  135                  140  
 Phe Xaa  
 145

<210> 107  
 <211> 80  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (80)

<223> Xaa equals stop translation

<400> 107

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Met Leu Arg Leu Thr Gln Thr Phe Phe Phe Ile Ser Gln Thr Leu Leu
 1             5             10             15

Asp Trp Phe Leu Ala Ala Ala Leu Ala Leu Pro Asn Leu Cys Ser Pro
          20             25             30

Leu Ala Ser Asn Phe Lys Ser Arg Gln Ile Ser Ser Val Pro Ile Gln
          35             40             45

Pro Ser Gln Gly Thr Ser Arg Val Ala Leu Gln Ile Trp Cys Gly Ser
          50             55             60

Cys Arg Met Arg Met Ser Ser Ser Thr Ile His Ile Leu Ala Leu Xaa
65             70             75             80

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<210> 108

<211> 83

<212> PRT

<213> Homo sapiens

<220>

<221> SITE

<222> (83)

<223> Xaa equals stop translation

<400> 108

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Met Leu Leu Leu Gln Ser Leu Phe Phe Pro Met Ser Trp Gly Ser Gly
 1             5             10             15

Gly Gly Gly Lys Gly Arg Asp Asp Leu Pro Arg Glu Lys Pro Thr Thr
          20             25             30

Cys Pro Val Phe Asp Arg Leu Phe Asp Ile Phe Ala Lys Ile Pro Leu
          35             40             45

Val Glu Ser Gln Ala Ser Cys Ala Arg Ile Gly Ile Ala Ala Ser His
          50             55             60

Trp Arg Leu Asp Cys Ser Val Asp Gly Met Gln Ala Asp Cys Leu Ser
65             70             75             80

Leu Ile Xaa

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<210> 109  
 <211> 115  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (115)  
 <223> Xaa equals stop translation

<400> 109  
 Met Val Thr Arg Ala Gly Ala Gly Thr Ala Val Ala Gly Ala Val Val  
     1                    5                    10                    15  
  
 Val Ala Leu Leu Ser Ala Ala Leu Ala Leu Tyr Gly Pro Pro Leu Asp  
                     20                    25                    30  
  
 Ala Val Leu Glu Arg Ala Phe Ser Leu Arg Lys Ala His Ser Ile Lys  
                     35                    40                    45  
  
 Asp Met Glu Asn Thr Leu Gln Leu Val Arg Asn Ile Ile Pro Pro Leu  
                     50                    55                    60  
  
 Ser Ser Thr Lys His Lys Gly Gln Asp Gly Arg Ile Gly Val Val Gly  
     65                    70                    75                    80  
  
 Gly Cys Gln Glu Tyr Thr Gly Ala Pro Tyr Phe Ala Glu Ser Gln Leu  
                     85                    90                    95  
  
 Ser Lys Trp Ala Gln Thr Cys Pro Thr Cys Ser Val Pro Val Arg Pro  
                     100                    105                    110  
  
 His Leu Xaa  
             115

<210> 110  
 <211> 164  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (164)  
 <223> Xaa equals stop translation

<400> 110  
 Met Ser Ser Arg Leu Ile Tyr Thr Leu Arg Cys Gly Val Phe Ala Thr  
     1                    5                    10                    15

90

Phe Pro Ile Val Leu Gly Ile Leu Val Tyr Gly Leu Ser Leu Leu Cys  
                   20                  25                  30  
 Phe Ser Ala Leu Arg Pro Phe Gly Glu Pro Arg Arg Glu Val Glu Ile  
                   35                  40                  45  
 His Arg Arg Tyr Val Ala Gln Ser Val Gln Leu Phe Ile Leu Tyr Phe  
                   50                  55                  60  
 Phe Asn Leu Ala Val Leu Ser Thr Tyr Leu Pro Gln Asp Thr Leu Lys  
                   65                  70                  75                  80  
 Leu Leu Pro Leu Leu Thr Gly Leu Phe Ala Val Ser Arg Leu Ile Tyr  
                   85                  90                  95  
 Trp Leu Thr Phe Ala Val Gly Arg Ser Phe Arg Gly Phe Gly Tyr Gly  
                   100                  105                  110  
 Leu Thr Phe Leu Pro Leu Leu Ser Met Leu Met Trp Asn Leu Tyr Tyr  
                   115                  120                  125  
 Met Phe Val Val Glu Pro Glu Arg Met Leu Thr Ala Thr Glu Ser Arg  
                   130                  135                  140  
 Leu Asp Tyr Pro Asp His Ala Arg Ser Ala Ser Asp Tyr Arg Pro Arg  
                   145                  150                  155                  160  
 Pro Trp Gly Xaa

&lt;210&gt; 111

&lt;211&gt; 259

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (259)

&lt;223&gt; Xaa equals stop translation

&lt;400&gt; 111

Met Tyr Ile Trp Phe Ile Ile Phe Phe Ile Gln Pro His Lys Glu Glu  
           1                  5                  10                  15  
 Arg Phe Leu Phe Pro Val Tyr Pro Leu Ile Cys Leu Cys Gly Ala Val  
                   20                  25                  30  
 Ala Leu Ser Ala Leu Gln Lys Cys Tyr His Phe Val Phe Gln Arg Tyr  
                   35                  40                  45

Arg Leu Glu His Tyr Thr Val Thr Ser Asn Trp Leu Ala Leu Gly Thr  
 50 55 60  
 Val Phe Leu Phe Gly Leu Leu Ser Phe Ser Arg Ser Val Ala Leu Phe  
 65 70 75 80  
 Arg Gly Tyr His Gly Pro Leu Asp Leu Tyr Pro Glu Phe Tyr Arg Ile  
 85 90 95  
 Ala Thr Asp Pro Thr Ile His Thr Val Pro Glu Gly Arg Pro Val Asn  
 100 105 110  
 Val Cys Val Gly Lys Glu Trp Tyr Arg Phe Pro Ser Ser Phe Leu Leu  
 115 120 125  
 Pro Asp Asn Trp Gln Leu Gln Phe Ile Pro Ser Glu Phe Arg Gly Gln  
 130 135 140  
 Leu Pro Lys Pro Phe Ala Glu Gly Pro Leu Ala Thr Arg Ile Val Pro  
 145 150 155 160  
 Thr Asp Met Asn Asp Gln Asn Leu Glu Glu Pro Ser Arg Tyr Ile Asp  
 165 170 175  
 Ile Ser Lys Cys His Tyr Leu Val Asp Leu Asp Thr Met Arg Glu Thr  
 180 185 190  
 Pro Arg Glu Pro Lys Tyr Ser Ser Asn Lys Glu Glu Trp Ile Ser Leu  
 195 200 205  
 Ala Tyr Arg Pro Phe Leu Asp Ala Ser Arg Ser Ser Lys Leu Leu Arg  
 210 215 220  
 Ala Phe Tyr Val Pro Phe Leu Ser Asp Gln Tyr Thr Val Tyr Val Asn  
 225 230 235 240  
 Tyr Thr Ile Leu Lys Pro Arg Lys Ala Lys Gln Ile Arg Lys Lys Ser  
 245 250 255  
 Gly Gly Xaa

&lt;210&gt; 112

&lt;211&gt; 97

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (97)

<223> Xaa equals stop translation

<400> 112

Met Ala Arg Ala Cys Val Phe Gln Leu Ser Leu Trp Arg Lys Leu Pro  
1 5 10 15

Val Gly Ile Asn Leu Ser Pro Ala Ile Leu Ser Leu Ser Leu Gly Cys  
20 25 30

Leu Gly Leu Gly Phe Leu Leu Leu Leu Glu Arg Met Thr Thr Asp Ser  
35 40 45

Gly Ile Arg Gln Arg Ser Arg His Asp Leu Leu Gly Phe Cys Gly Cys  
50 55 60

Gln His Cys Arg Ser Phe Trp Arg Leu Arg Glu Ala Leu Glu Gly Ile  
65 70 75 80

Gly Thr Ser Cys Cys Arg Pro Pro Gly Arg Ala Gly Leu Phe Ile Phe  
85 90 95

Xaa

<210> 113

<211> 73

<212> PRT

<213> Homo sapiens

<220>

<221> SITE

<222> (73)

<223> Xaa equals stop translation

<400> 113

Met Arg His Thr Cys Ile Val Asn Ile Ala Ala Ser Leu Leu Val Ala  
1 5 10 15

Asn Thr Trp Phe Ile Val Val Ala Ala Ile Gln Asp Asn Arg Tyr Ile  
20 25 30

Leu Cys Lys Thr Ala Cys Val Ala Ala Thr Phe Phe Ile His Phe Phe  
35 40 45

Tyr Leu Ser Val Phe Phe Trp Met Leu Thr Leu Gly Pro His Ala Val  
50 55 60

Leu Ser Pro Gly Phe His Ser Ala Xaa  
65 70

<210> 114  
 <211> 276  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (276)  
 <223> Xaa equals stop translation

<400> 114  
 Met Thr Ile Thr Ser Phe Tyr Ala Val Cys Phe Tyr Leu Leu Met Leu  
 1 5 10 15  
 Val Met Val Glu Gly Phe Gly Gly Lys Glu Ala Val Leu Arg Thr Leu  
 20 25 30  
 Arg Asp Thr Pro Met Met Val His Thr Gly Pro Cys Cys Cys Cys Cys  
 35 40 45  
 Pro Cys Cys Pro Arg Leu Leu Leu Thr Arg Lys Lys Leu Gln Leu Leu  
 50 55 60  
 Met Leu Gly Pro Phe Gln Tyr Ala Phe Leu Lys Ile Thr Leu Thr Leu  
 65 70 75 80  
 Val Gly Leu Phe Leu Ile Pro Asp Gly Ile Tyr Asp Pro Ala Asp Ile  
 85 90 95  
 Ser Glu Gly Ser Thr Ala Leu Trp Ile Asn Thr Phe Leu Gly Val Ser  
 100 105 110  
 Thr Leu Leu Ala Leu Trp Thr Leu Gly Ile Ile Ser Arg Gln Ala Arg  
 115 120 125  
 Leu His Leu Gly Glu Gln Asn Met Gly Ala Lys Phe Ala Leu Phe Gln  
 130 135 140  
 Val Leu Leu Ile Leu Thr Ala Leu Gln Pro Ser Ile Phe Ser Val Leu  
 145 150 155 160  
 Ala Asn Gly Gly Gln Ile Ala Cys Ser Pro Pro Tyr Ser Ser Lys Thr  
 165 170 175  
 Arg Ser Gln Val Met Asn Cys His Leu Leu Ile Leu Glu Thr Phe Leu  
 180 185 190  
 Met Thr Val Leu Thr Arg Met Tyr Tyr Arg Arg Lys Asp His Lys Val  
 195 200 205



Gly Tyr Glu Thr Phe Ser Ser Pro Asp Leu Asp Leu Asn Ser Lys Pro  
 210 215 220

Lys Val Asp Gly Leu Asp Asn Glu Arg Met Leu Tyr Ser Leu Glu Tyr  
 225 230 235 240

Lys Ile Pro Leu Leu Ser Leu Asn Leu Asp Gln Met Gly Ser Ile Pro  
 245 250 255

Pro Cys Gln His Lys Leu Ala Asp Thr Phe Asp Ser Thr Asp Glu Gly  
 260 265 270

Glu Gln Cys Xaa  
 275

<210> 115

<211> 627

<212> PRT

<213> Homo sapiens

<400> 115

Met Glu Ala Arg Val Val His Ala Leu Gln Lys Arg Gln Val Ser Leu  
 1 5 10 15

Leu Cys Val Phe Leu Gly Val Ser Trp Ala Gly Ala Glu Pro Leu Arg  
 20 25 30

Tyr Phe Val Ala Glu Glu Thr Glu Arg Gly Thr Phe Leu Ala Asn Leu  
 35 40 45

Ala Ile Asp Leu Gly Leu Gly Val Glu Glu Leu Ser Ala Arg Gly Cys  
 50 55 60

Arg Ile Val Ser Asp Glu Thr Ile Gly Phe Leu Leu Leu Asn Pro Leu  
 65 70 75 80

Thr Gly Asp Leu Leu Leu Asn Glu Lys Leu Asp Arg Glu Glu Leu Cys  
 85 90 95

Gly Pro Thr Glu Pro Cys Val Leu Pro Phe Gln Leu Leu Leu Glu Lys  
 100 105 110

Pro Phe Gln Ile Phe Arg Ala Glu Leu Trp Val Arg Asp Ile Asn Asp  
 115 120 125

His Ser Pro Val Phe Leu Asp Arg Glu Ile Thr Leu Asn Ile Leu Glu  
 130 135 140

Ser Thr Thr Pro Gly Ala Thr Phe Leu Leu Glu Ser Ala His Asp Ser  
 145 150 155 160

Asp Val Gly Ile Asn Asn Leu Arg Asn Tyr Thr Ile Ser Ser Asn Val  
 165 170 175

Tyr Phe His Ile Asn Val His Asp Asn Gly Glu Gly Asn Val Tyr Ser  
 180 185 190

Glu Leu Val Leu Asp Lys Val Leu Asp Arg Glu Glu Val Pro Glu Leu  
 195 200 205

Arg Leu Thr Leu Thr Gly Leu Asp Gly Gly Ser Pro Pro Arg Ser Gly  
 210 215 220

Thr Thr Leu Ile Arg Ile Leu Val Leu Asp Ile Asn Asp Asn Val Pro  
 225 230 235 240

Glu Phe Val Glu Ser Leu Tyr Lys Val Gln Val Pro Glu Asn Ser Pro  
 245 250 255

Val Gly Ser Leu Val Val Thr Val Ser Ala Arg Asp Leu Asp Thr Gly  
 260 265 270

Ser Asn Gly Glu Ile Val Tyr Ala Phe Phe Tyr Ala Thr Glu Arg Thr  
 275 280 285

Leu Lys Thr Phe Arg Ile Asn Ser Thr Ser Gly Asn Leu His Leu Lys  
 290 295 300

Ala Glu Leu Asn Tyr Glu Ala Ile Gln Thr Tyr Thr Leu Thr Ile Gln  
 305 310 315 320

Ala Lys Asp Gly Gly Gly Leu Ser Gly Lys Cys Thr Val Val Val His  
 325 330 335

Val Thr Asp Ile Asn Asp Asn Pro Pro Glu Leu Leu Met Ser Ser Leu  
 340 345 350

Thr Ser Pro Ile Pro Glu Asn Ser Pro Glu Thr Val Val Ala Val Phe  
 355 360 365

Arg Ile Arg Asp Arg Asp Ser Gly Asn Asn Ala Lys Met Val Cys Ser  
 370 375 380

Ile Gln Asp His Leu Pro Phe Val Leu Lys Pro Ser Val Glu Asn Phe  
 385 390 395 400

Tyr Thr Leu Val Thr Glu Arg Ala Leu Asp Arg Glu Glu Arg Thr Glu  
 405 410 415

Tyr Asn Ile Thr Ile Thr Val Thr Asp Leu Gly Thr Pro Arg Leu Lys  
 420 425 430

Thr Gln His Asn Leu Thr Val Thr Val Ser Asp Val Asn Asp Asn Ala  
 435 440 445  
 Pro Thr Phe Ser Gln Thr Thr Tyr Thr Leu Arg Val Arg Glu Asn Asn  
 450 455 460  
 Ser Pro Ala Leu His Ile Gly Ser Val Ser Ala Thr Asp Arg Asp Ser  
 465 470 475 480  
 Gly Ala Asn Ala Gln Val Thr Tyr Ser Leu Leu Pro Pro His Asp Pro  
 485 490 495  
 Gln Leu Pro Leu Gly Ser Leu Val Ser Ile Asn Ala Asp Asn Gly Gln  
 500 505 510  
 Leu Phe Ala Leu Arg Ser Leu Asp Phe Glu Ala Leu Gln Ala Phe Glu  
 515 520 525  
 Phe Arg Val Gly Ala Ala Asp Arg Gly Ser Pro Ala Leu Ser Ser Gln  
 530 535 540  
 Ala Leu Val Arg Val Leu Val Ala Asp Ala Asn Asp Asn Ala Pro Phe  
 545 550 555 560  
 Val Leu Tyr Pro Leu Gln Asn Gly Ser Ala Pro Cys Thr Glu Leu Val  
 565 570 575  
 Pro Arg Ala Ala Glu Ala Gly Tyr Leu Val Ala Lys Val Val Ala Val  
 580 585 590  
 Asp Gly Asp Ser Gly Gln Asn Ala Trp Leu Ser Tyr Gln Leu Leu Lys  
 595 600 605  
 Ala Thr Glu Pro Gly Leu Phe Gly Val Trp Ala His Asn Gly Glu Val  
 610 615 620  
 Arg Thr Ala  
 625

&lt;210&gt; 116

&lt;211&gt; 52

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (52)

&lt;223&gt; xaa equals stop translation

97

&lt;400&gt; 116

Met Arg Ala Val His Pro Ala Leu Gly Leu Cys Leu Leu Pro Ala Pro  
1 5 10 15

Ser Cys Gly Lys Val Leu Val Ala Gly Ala Leu Glu Gly Val Pro Ala  
20 25 30

Gly Val Ala Glu Ala Glu Ala Asn Ile Ala Gln Val Pro Pro Ile Ala  
35 40 45

Arg Gln Thr Xaa  
50

&lt;210&gt; 117

&lt;211&gt; 75

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (75)

&lt;223&gt; Xaa equals stop translation

&lt;400&gt; 117

Met Phe Thr Gly Leu Leu Ile Tyr Leu Leu Val Ser Ser Ile Leu Ile  
1 5 10 15

Ser Leu Ala Asp Arg Pro Phe Ser Ser Ile Arg Cys Leu Thr Phe Trp  
20 25 30

Val Gln Phe Ile Arg Leu Cys Tyr Ile Arg Asn Thr Ser Leu Leu Pro  
35 40 45

Met Thr Cys Val Ala Tyr Ile Phe Phe Leu Phe Tyr Phe Phe Thr Ile  
50 55 60

Gln Lys Phe Leu Val Lys Ile Ile Asn Phe Xaa  
65 70 75

&lt;210&gt; 118

&lt;211&gt; 120

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 118

Met Leu Pro Ala Leu Arg Gly Leu Leu Phe Val Thr Trp Val Phe Pro  
1 5 10 15

Leu Glu Asp Gln Glu Ala Ala Ala Phe Pro Gly Glu Val Asp Pro Pro

98

20                                      25                                      30  
 Ser Pro Phe Gly Pro Cys Thr Ala Glu Gly Pro Ala Ala Leu Pro Ala  
                  35                                      40                                      45  
 Arg Val Trp Ser Val Lys Gln Gly Leu Arg Pro Phe Ser Cys Ser Asp  
                  50                                      55                                      60  
 Ala Pro Gln Gly Asp Ser Arg Glu Leu Ala Lys Pro Pro Gly Leu Pro  
                  65                                      70                                      75                                      80  
 Pro Val Arg Gly Ala Leu Val Thr Trp Pro Pro Pro Gln Pro Thr Gly  
    85                                      90                                      95  
 Leu Ser Arg Leu Arg Cys His Pro His Gly Thr Gly Gly Asn His Ser  
    100                                      105                                      110  
 Ile Arg Cys Arg Arg Cys Arg Pro  
    115                                      120

<210> 119  
 <211> 264  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (264)  
 <223> Xaa equals stop translation

<400> 119  
 Met Pro Arg Arg Pro Ser Cys Pro Leu Gly Cys Trp Ser Leu Leu Leu  
                  1                                      5                                      10                                      15  
 Gly Leu Ser Ser Leu Ser Leu Pro Ala Ala Ile Ser Ala Leu Gln Leu  
    20                                      25                                      30  
 Ser Val Phe Arg Lys Glu Pro Ser Pro Gln Asn Gly Asn Ile Thr Ala  
    35                                      40                                      45  
 Gln Gly Pro Ser Ile Gln Pro Val His Lys Ala Glu Ser Ser Thr Asp  
    50                                      55                                      60  
 Ser Ser Gly Pro Leu Glu Glu Ala Glu Glu Ala Pro Gln Leu Met Arg  
    65                                      70                                      75                                      80  
 Thr Lys Ser Asp Ala Ser Cys Met Ser Gln Arg Arg Pro Lys Cys Arg  
    85                                      90                                      95  
 Ala Pro Gly Glu Ala Gln Arg Ile Arg Arg His Arg Phe Ser Ile Asn

100	105	110
Gly His Phe Tyr Asn His Lys Thr Ser Val Phe Thr Pro Ala Tyr Gly		
115	120	125
Ser Val Thr Asn Val Arg Val Asn Ser Thr Met Thr Thr Leu Gln Val		
130	135	140
Leu Thr Leu Leu Leu Asn Lys Phe Arg Val Glu Asp Gly Pro Ser Glu		
145	150	155
Phe Ala Leu Tyr Ile Val His Glu Ser Gly Glu Arg Thr Lys Leu Lys		
165	170	175
Asp Cys Glu Tyr Pro Leu Ile Ser Arg Ile Leu His Gly Pro Cys Glu		
180	185	190
Lys Ile Ala Arg Ile Phe Leu Met Glu Ala Asp Leu Gly Val Glu Val		
195	200	205
Pro His Glu Val Ala Gln Tyr Ile Lys Phe Glu Met Pro Val Leu Asp		
210	215	220
Ser Phe Val Glu Lys Leu Lys Glu Glu Glu Glu Arg Glu Ile Ile Lys		
225	230	235
Leu Thr Met Lys Phe Gln Ala Leu Arg Leu Thr Met Leu Gln Arg Leu		
245	250	255
Glu Gln Leu Val Glu Ala Lys Xaa		
260		

&lt;210&gt; 120

&lt;211&gt; 96

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (96)

&lt;223&gt; Xaa equals stop translation

&lt;400&gt; 120

Met Ser Ala Trp Leu Val Ser Leu Cys Ala Trp Leu Ser Leu Leu Arg
1 5 10 15

Ala Thr Val Thr Ser Gln Val Ser Ser Ser Pro Ala Pro Val Val Ala
20 25 30

Ser Gly Thr Leu Ser Pro Cys His Pro Pro Gly Ser Pro Ala Ala Ser

100

35 40 45  
Ala Cys Leu Leu Ser Pro Gln Ser Pro Cys Arg Arg Ala Ser Lys Trp  
50 55 60  
Arg Ser His Met Thr Gly Val Ala Pro Ser Asn Arg Gly Ser Ser Cys  
65 70 75 80  
Glu Ser Ser Gly Ser Gln Gly Lys Pro Ser Gln Arg Ala Gly Ala Xaa  
85 90 95

<210> 121  
<211> 61  
<212> PRT  
<213> Homo sapiens

<220>  
<221> SITE  
<222> (61)  
<223> Xaa equals stop translation

<400> 121  
Met His Ile Pro Leu Trp Pro Asn Trp Leu Leu Phe Val Cys Lys Leu  
1 5 10 15  
Leu Phe Leu Ser His Pro Ile Leu Leu Ala Cys Val Lys Cys Lys Ser  
20 25 30  
Gln Val Phe Pro Ala Gly Ser Asn Val Phe Leu Ser Leu Asn Gln Gly  
35 40 45  
Pro Thr Gly Cys Leu Leu Leu Gln Ile Lys Phe Tyr Xaa  
50 55 60

<210> 122  
<211> 158  
<212> PRT  
<213> Homo sapiens

<220>  
<221> SITE  
<222> (158)  
<223> Xaa equals stop translation

<400> 122  
Met Val Lys Ser Val Ile Phe Leu Ser Phe Trp Gln Gly Met Leu Leu

101

1	5	10	15
Ala Ile Leu Glu Lys Cys Gly Ala Ile Pro Lys Ile His Ser Ala Arg	20	25	30
Val Ser Val Gly Glu Gly Thr Val Ala Ala Gly Tyr His Asp Phe Ile	35	40	45
Ile Cys Val Glu Met Phe Phe Ala Ala Leu Ala Leu Arg His Pro Phe	50	55	60
Thr Tyr Asn Val Tyr Ala Asp Lys Arg Leu Asp Ala Gln Gly Arg Cys	65	70	75
Ala Pro Met Lys Ser Ile Ser Ser Ser Leu Lys Glu Thr Met Asn Pro	85	90	95
His Asp Ile Val Gln Asp Ala Ile His Asn Phe Ser Pro Ala Tyr Gln	100	105	110
Gln Tyr Thr Gln Gln Ser Thr Leu Glu Pro Gly Pro Thr Trp Arg Gly	115	120	125
Gly Ala His Gly Leu Ser Arg Ser His Ser Leu Ser Gly Ala Arg Asp	130	135	140
Asn Glu Lys Thr Leu Leu Leu Ser Ser Asp Asp Glu Phe Xaa	145	150	155

<210> 123

<211> 116

<212> PRT

<213> Homo sapiens

**<220>**

**<221> SITE**

**<222> (116)**

<223> Xaa equals stop translation

<400> 123

Met	Ser	Asp	Phe	Ser	Asn	Leu	Ser	Leu	Leu	Phe	Phe	Leu	Leu	Val	Ser
1				5					10					15	
Leu	Ala	Lys	Gly	Leu	Ser	Ile	Leu	Phe	Ile	Tyr	Ser	Glu	Asn	His	Leu
			20					25					30		
Leu	Val	Leu	Phe	Ile	Phe	Leu	Ile	Phe	Lys	Glu	Thr	Thr	Arg	Pro	Ala
		35					40					45			
Ala	Phe	Cys	Val	Ser	Val	Glu	Ser	Cys	Tyr	Gly	Ser	Gly	Ser	Cys	Leu



102

50                      55                      60

Ser Ser Leu Ser Val Glu Trp Pro Gly Gln Cys Met Trp Arg Leu Leu  
65                      70                      75                      80

Arg Leu Pro Phe Thr Arg Val Ala Leu Pro Leu Pro Val Trp His Phe  
85                      90                      95

His Val Thr Phe Leu Leu Lys Ser Trp Phe Thr Ala Lys Val Leu Ala  
100                      105                      110

Phe Ile Gln Xaa  
115

<210> 124  
<211> 91  
<212> PRT  
<213> Homo sapiens

<220>  
<221> SITE  
<222> (39)  
<223> Xaa equals any of the naturally occurring L-amino acids

<220>  
<221> SITE  
<222> (45)  
<223> Xaa equals any of the naturally occurring L-amino acids

<220>  
<221> SITE  
<222> (49)  
<223> Xaa equals any of the naturally occurring L-amino acids

<220>  
<221> SITE  
<222> (51)  
<223> Xaa equals any of the naturally occurring L-amino acids

<220>  
<221> SITE  
<222> (62)  
<223> Xaa equals any of the naturally occurring L-amino acids

<220>  
<221> SITE  
<222> (64)  
<223> Xaa equals any of the naturally occurring L-amino acids

<220>

103

&lt;221&gt; SITE

&lt;222&gt; (91)

&lt;223&gt; Xaa equals stop translation

&lt;400&gt; 124

Met Gly Ile Trp Val Leu Ala Leu Trp Val Gly Cys Leu Cys Phe Leu  
1 5 10 15

Tyr Arg Pro Ala Cys Gly Thr Asp Gln Cys Gly Ala Trp Ser Lys Val  
20 25 30

Arg Arg Thr Ala Met Ala Xaa Ala Thr Gly Ala Ala Xaa Ser Thr Pro  
35 40 45

Xaa Ala Xaa Trp Leu Leu Ser Val Ser His Thr Thr Leu Xaa Leu Xaa  
50 55 60

Ala Met Glu Lys Gly Glu Ala Gln Arg Ala Asn Cys Gln His Ser Cys  
65 70 75 80

Val Asp Thr Leu Gly Pro Gln His Gln Pro Xaa  
85 90

&lt;210&gt; 125

&lt;211&gt; 97

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (5)

&lt;223&gt; Xaa equals any of the naturally occurring L-amino acids

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (8)

&lt;223&gt; Xaa equals any of the naturally occurring L-amino acids

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (14)

&lt;223&gt; Xaa equals any of the naturally occurring L-amino acids

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (97)

&lt;223&gt; Xaa equals stop translation

&lt;400&gt; 125

Met Arg Val Thr Xaa Ala Thr Xaa Ala Leu Leu Leu Ala Xaa Ile Cys

104

1                      5                      10                      15  
 Ser Val Gln Leu Gly Asp Ala Cys Leu Asp Ile Asp Lys Leu Leu Ala  
                     20                      25                      30  
 Asn Val Val Phe Asp Val Ser Gln Asp Leu Leu Lys Glu Glu Leu Ala  
                     35                      40                      45  
 Arg Tyr Asn Pro Ser Pro Leu Thr Glu Glu Ser Phe Leu Asn Val Gln  
                     50                      55                      60  
 Gln Cys Phe Ala Asn Val Ser Val Thr Glu Arg Phe Ala His Ser Val  
                     65                      70                      75                      80  
 Val Ile Lys Lys Ile Leu Gln Ser Asn Asp Cys Ile Glu Ala Ala Phe  
                     85                      90                      95

Xaa

<210> 126  
 <211> 44  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (44)  
 <223> Xaa equals stop translation

<400> 126  
 Met Leu Val Ser Ser Pro Phe Ser Ser Pro Val Ser Phe Trp Ala Val  
                     1                      5                      10                      15  
 Phe Val Cys Leu Leu Leu Tyr Lys Ile Arg Thr Val Asn Tyr Leu  
                     20                      25                      30  
 Leu Cys Arg Ser Pro Ala Phe His Ser Ala Leu Xaa  
                     35                      40

<210> 127  
 <211> 42  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (42)  
 <223> Xaa equals stop translation

105

&lt;400&gt; 127

Met Glu Pro Cys Leu Ala Val Ala Leu Ser Val Tyr Ile Trp Leu Arg  
1 5 10 15

Ala Thr Ser Ala Lys Leu Leu Pro Asp Leu Asn Glu Ser Ala Glu Ile  
20 25 30

Ile Gly Pro Ser Ala Ala Glu Lys Lys Xaa  
35 40

&lt;210&gt; 128

&lt;211&gt; 53

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (53)

&lt;223&gt; Xaa equals stop translation

&lt;400&gt; 128

Met Lys Cys Phe Phe Leu Phe Val Val Ile Leu Ile Ile Met Lys Ser  
1 5 10 15

Asn Leu Ser Asp Ile Ile Ile Ala Thr Tyr Thr Tyr Cys Ile Pro Asp  
20 25 30

Tyr Phe Phe His Thr Phe Ile Phe Asn Leu Ser Val Tyr Leu Asn Ser  
35 40 45

Lys Phe Ile Ser Xaa  
50

&lt;210&gt; 129

&lt;211&gt; 44

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (44)

&lt;223&gt; Xaa equals stop translation

&lt;400&gt; 129

Met Ile Val Tyr Tyr Leu Ala Phe Phe Gly Leu Leu Asp Leu Cys Leu  
1 5 10 15

Gly Glu Gly Asn Phe Ser Ala Arg Glu Ala Val Trp Val Ile Cys Phe

106

20 25 30

Phe Ala Arg Asp Tyr Ser Pro Lys Tyr Tyr Arg Xaa  
35 40

<210> 130  
<211> 49  
<212> PRT  
<213> Homo sapiens

<220>  
<221> SITE  
<222> (49)  
<223> Xaa equals stop translation

<400> 130  
Met Ile Leu Gly Leu Leu Asn Leu Leu Arg Ile Val Val Phe Leu Ile  
1 5 10 15

Ala Trp Ser Ile Leu Glu Tyr Val Thr His Gly Asp Glu Lys Asp Ile  
20 25 30

Tyr Thr Met Leu Val Ser Asp Glu Glu Phe His Ile Cys Leu Leu Glu  
35 40 45

Xaa

<210> 131  
<211> 410  
<212> PRT  
<213> Homo sapiens

<220>  
<221> SITE  
<222> (78)  
<223> Xaa equals any of the naturally occurring L-amino acids

<220>  
<221> SITE  
<222> (168)  
<223> Xaa equals any of the naturally occurring L-amino acids

<400> 131  
Met Asn Pro Ala Val Arg Gln Arg Cys Leu Leu Phe Cys Phe Gln Gln  
1 5 10 15

Lys Leu Ile Leu Ser His Phe Phe Leu Leu Gln Val Pro Gln Trp Cys  
20 25 30

Ala Glu Tyr Cys Leu Ser Ile His Tyr Gln His Gly Gly Val Ile Cys  
 35 40 45

Thr Gln Val His Lys Gln Thr Val Val Gln Leu Ala Leu Arg Val Ala  
 50 55 60

Asp Glu Met Asp Val Asn Ile Gly His Glu Val Gly Tyr Xaa Ile Pro  
 65 70 75 80

Phe Glu Asn Cys Cys Thr Asn Glu Thr Ile Leu Arg Tyr Cys Thr Asp  
 85 90 95

Asp Met Leu Gln Arg Glu Met Met Ser Asn Pro Phe Leu Gly Ser Tyr  
 100 105 110

Gly Val Ile Ile Leu Asp Asp Ile His Glu Arg Ser Ile Ala Thr Asp  
 115 120 125

Val Leu Leu Gly Leu Leu Lys Asp Val Leu Leu Ala Arg Pro Glu Leu  
 130 135 140

Lys Leu Ile Ile Asn Ser Ser Pro His Leu Ile Ser Lys Leu Asn Ser  
 145 150 155 160

Tyr Tyr Gly Asn Val Pro Val Xaa Glu Val Lys Asn Lys His Pro Val  
 165 170 175

Glu Val Val Tyr Leu Ser Glu Ala Gln Lys Asp Ser Phe Glu Ser Ile  
 180 185 190

Leu Arg Leu Ile Phe Glu Ile His His Ser Gly Glu Lys Gly Asp Ile  
 195 200 205

Val Val Phe Leu Ala Cys Glu Gln Asp Ile Glu Lys Val Cys Glu Thr  
 210 215 220

Val Tyr Gln Gly Ser Asn Leu Asn Pro Asp Leu Gly Glu Leu Val Val  
 225 230 235 240

Val Pro Leu Tyr Pro Lys Glu Lys Cys Ser Leu Phe Lys Pro Leu Asp  
 245 250 255

Glu Thr Glu Lys Arg Cys Gln Val Tyr Gln Arg Arg Val Val Leu Thr  
 260 265 270

Thr Ser Ser Gly Glu Phe Leu Ile Trp Ser Asn Ser Val Arg Phe Val  
 275 280 285

Ile Asp Val Gly Val Glu Arg Arg Lys Val Tyr Asn Pro Arg Ile Arg  
 290 295 300

108

Ala Asn Ser Leu Val Met Gln Pro Ile Ser Gln Ser Gln Ala Glu Ile  
305 310 315 320

Arg Lys Gln Ile Leu Gly Ser Ser Ser Ser Gly Lys Phe Phe Cys Leu  
325 330 335

Tyr Thr Glu Glu Phe Ala Ser Lys Asp Met Thr Pro Leu Lys Pro Ala  
340 345 350

Glu Met Gln Glu Ala Asn Leu Thr Ser Met Val Leu Phe Met Lys Arg  
355 360 365

Ile Asp Ile Ala Gly Leu Gly His Cys Asp Phe Met Asn Arg Pro Gly  
370 375 380

Ser Leu Met Leu Pro Cys Gln Pro Gly Ile Arg Leu Arg Phe Thr Phe  
385 390 395 400

Ser Cys Pro Phe Ser Val Leu Ser Ser His  
405 410

&lt;210&gt; 132

&lt;211&gt; 65

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (65)

&lt;223&gt; Xaa equals stop translation

&lt;400&gt; 132

Met Leu Arg Phe Leu Gly Asn Gln Met Tyr Ala Leu Tyr Thr Trp Leu  
1 5 10 15

Leu Leu Gln Ser Pro Val Cys Ser Ala Val Leu Val Thr Ser Ala Leu  
20 25 30

Leu Tyr Pro Ser Leu Leu Thr Leu Arg Pro Ser Gln Ala His Ala Ala  
35 40 45

Cys Ile Tyr Leu Pro Ser Val Ser Leu Val Ser Leu Ser Asp Pro Phe  
50 55 60

Xaa

65

&lt;210&gt; 133

109

<211> 44  
<212> PRT  
<213> Homo sapiens

<220>  
<221> SITE  
<222> (44)  
<223> Xaa equals stop translation

<400> 133  
Met Asn Leu Ile Phe Arg Leu Pro Cys Ile Leu Leu Thr Cys Ile Tyr  
1 5 10 15  
Val Gln Gln Cys Val Cys Lys Tyr Ile Gly Thr Phe Leu Asn Arg Val  
20 25 30  
Cys Ala Met Cys Lys Gly Leu Leu Thr Val Lys Xaa  
35 40

<210> 134  
<211> 59  
<212> PRT  
<213> Homo sapiens

<220>  
<221> SITE  
<222> (59)  
<223> Xaa equals stop translation

<400> 134  
Met Val Ser Phe Gly Phe Trp Phe Leu Cys Leu Phe Phe Gly Val Trp  
1 5 10 15  
Lys Asn Met His Phe Tyr Arg Ala Arg Lys Leu Val Ser Arg Lys Gly  
20 25 30  
Ser Pro Glu Lys Ala Ala Asp Gly Pro Cys Pro Cys Trp Val Phe Leu  
35 40 45  
Phe Phe Gly Thr Val Arg Gly Asn Gly Phe Xaa  
50 55

<210> 135  
<211> 104  
<212> PRT  
<213> Homo sapiens

<220>  
<221> SITE



110

&lt;222&gt; (104)

&lt;223&gt; Xaa equals stop translation

&lt;400&gt; 135

Met Ala His Ile Gly Ala Cys Val Ser Phe Val Phe Phe Leu Leu Gln  
1 5 10 15

Gly Ala Val Ser Val Trp Thr Phe Cys Phe Arg Glu Leu Glu Arg Arg  
20 25 30

Val Ser Ala Glu Gly Gly Glu Gln Gly Gln Arg Pro His Trp Pro Pro  
35 40 45

Pro Ala Ser Gln Ser Glu Thr Leu Cys Leu Val Thr Lys Val Pro Pro  
50 55 60

Lys Cys Ser Ser Phe Trp Val Ile Gln Ala Lys Tyr Leu Gly Phe Pro  
65 70 75 80

Leu Ser Ser Phe Pro Ser Lys Pro Gln Leu Ser Phe Lys Ile Gly Asp  
85 90 95

Ile Ser His Pro Leu Pro Leu Xaa  
100

&lt;210&gt; 136

&lt;211&gt; 45

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (45)

&lt;223&gt; Xaa equals stop translation

&lt;400&gt; 136

Met Met Pro Leu Lys Leu His Ala Lys Cys Leu Tyr Leu Leu Lys Cys  
1 5 10 15

Val Phe Phe Val Gly Val Gly Gly Met Thr Phe Tyr Gln Ile Leu Thr  
20 25 30

Gly Phe Lys Ile Gln Lys Ser Leu Asp Leu Val Gly Xaa  
35 40 45

&lt;210&gt; 137

&lt;211&gt; 88

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

111

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (88)

&lt;223&gt; Xaa equals stop translation

&lt;400&gt; 137

Met Asp Leu Thr Val Glu Gly Phe Gln Ser Trp Met Trp Arg Gly Leu  
 1 5 10 15

Thr Phe Leu Leu Pro Phe Leu Phe Phe Gly His Phe Trp Gln Leu Phe  
 20 25 30

Asn Ala Leu Thr Leu Phe Asn Leu Ala Gln Asp Pro Gln Cys Lys Glu  
 35 40 45

Trp Gln Val Leu Met Cys Gly Phe Pro Phe Leu Leu Leu Phe Leu Gly  
 50 55 60

Asn Phe Phe Thr Thr Leu Arg Val Val His His Lys Phe His Ser Gln  
 65 70 75 80

Arg His Gly Ser Lys Lys Asp Xaa  
 85

&lt;210&gt; 138

&lt;211&gt; 66

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (66)

&lt;223&gt; Xaa equals stop translation

&lt;400&gt; 138

Met Ala Ser Pro Ser Ile Ile Leu Leu Leu Ile Phe Phe Phe Phe Phe  
 1 5 10 15

Phe Phe Ser Val Cys Ser Val Ser Gln Tyr Met Phe Glu Asn Glu Cys  
 20 25 30

Glu Ser Met Ser Arg Arg Arg Gly Arg Gly Leu Gly Arg Ser Arg Leu  
 35 40 45

Lys Val Glu Gln Gly Pro Asp Ala Asp Leu His Pro Arg Thr Leu Gly  
 50 55 60

Ser Xaa  
 65

112

<210> 139  
 <211> 88  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (88)  
 <223> Xaa equals stop translation

<400> 139  
 Met Thr Ala Trp Ile Leu Leu Pro Val Ser Leu Ser Ala Phe Ser Ile  
           1                  5                  10                  15  
 Thr Gly Ile Trp Thr Val Tyr Ala Met Ala Val Met Asn His His Val  
                   20                  25                  30  
 Cys Pro Val Glu Asn Trp Ser Tyr Asn Glu Ser Cys Pro Pro Asp Pro  
                   35                  40                  45  
 Ala Glu Gln Gly Gly Pro Lys Thr Cys Cys Thr Leu Asp Asp Val Pro  
           50                  55                  60  
 Leu Ile Ser Gly Pro Asp Leu Pro Pro Ala Leu Arg Ala Ala Pro Gly  
           65                  70                  75                  80  
 Ala Glu Ser Ala Leu Leu Gly Xaa  
                   85

<210> 140  
 <211> 57  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (57)  
 <223> Xaa equals stop translation

<400> 140  
 Met Lys Ile Pro Leu His Val Val Phe Leu Leu Ile Ser Leu Thr Phe  
           1                  5                  10                  15  
 Leu Phe Thr Thr Leu Pro Thr Ala His Ser Ala Pro Ser Ser Pro Ala  
                   20                  25                  30  
 Ser Leu His Ile Leu Arg Leu Arg Gly His Leu Met Cys Val Phe Pro  
           35                  40                  45

113

Leu Lys Met Met Pro Thr Leu Ile Xaa  
50 55

<210> 141  
<211> 46  
<212> PRT  
<213> Homo sapiens

<220>  
<221> SITE  
<222> (46)  
<223> Xaa equals stop translation

<400> 141  
Met Val Gln Trp Lys Asn Trp Pro Glu Ser Leu Glu Val Trp Val Leu  
1 5 10 15

Val Leu Ala Val Pro Leu Thr His Cys Asp Leu Gly Ile Leu Cys Cys  
20 25 30

Glu Asp Ile Ser Gln Val Leu His Val Ser Gln Gln Ile Xaa  
35 40 45

<210> 142  
<211> 53  
<212> PRT  
<213> Homo sapiens

<220>  
<221> SITE  
<222> (53)  
<223> Xaa equals stop translation

<400> 142  
Met Asp Ser Cys Leu Phe Leu Arg Asp Phe Cys Trp Lys Met Arg Met  
1 5 10 15

Leu Thr Ile Leu Pro Leu Gly Thr Leu Phe Pro Leu Leu Thr Leu Leu  
20 25 30

Leu Leu Pro Leu Glu Val Pro Ser Val Ser Cys Gly Val Pro Phe Ala  
35 40 45

Val Trp Asp Leu Xaa  
50

<210> 143

114

<211> 81  
<212> PRT  
<213> Homo sapiens

<220>  
<221> SITE  
<222> (81)  
<223> Xaa equals stop translation

&lt;400&gt; 143

Met Ala Leu Trp Val Thr Cys Ile Leu Ser Leu Cys Thr Trp Phe Ser  
1 5 10 15

Cys Leu Tyr Gly Ala Asp Ser Leu Ala Asn Lys Cys Leu Ser Ala Gly  
20 25 30

Ala Thr Arg Lys Ala Phe Pro Phe Cys Val Leu Phe Arg Asp Leu Glu  
35 40 45

Val Gly Leu Gly Phe Glu Gly Phe Val Thr His Leu Ala Cys Lys Leu  
50 55 60

Phe Cys Tyr Cys Glu Leu Ser Asp Ser Ala Leu Ser Leu Gly His Glu  
65 70 75 80

Xaa

<210> 144  
<211> 65  
<212> PRT  
<213> Homo sapiens

<220>  
<221> SITE  
<222> (65)  
<223> Xaa equals stop translation

&lt;400&gt; 144

Met Asn Ile Pro Trp Leu Tyr Phe Val Asn Ser Phe Leu Ile Ala Thr  
1 5 10 15

Val Tyr Trp Phe Asn Cys His Lys Leu Asn Leu Lys Asp Ile Gly Leu  
20 25 30

Pro Leu Asp Pro Phe Val Asn Trp Lys Cys Cys Phe Ile Pro Leu Thr  
35 40 45

Ile Pro Asn Leu Glu Gln Ile Glu Lys Pro Ile Ser Ile Met Ile Cys  
50 55 60

Xaa  
65

<210> 145  
<211> 52  
<212> PRT  
<213> Homo sapiens

<220>  
<221> SITE  
<222> (52)  
<223> Xaa equals stop translation

<400> 145  
Met Ser Phe Asp Ala Glu Lys Phe Leu Ile Leu Lys Phe Ile Leu Gln  
1 5 10 15

Phe Phe Leu Leu Leu Tyr Val Leu Phe Leu Val Leu Tyr Leu Arg Ile  
20 25 30

Cys Cys His Thr Gln Gly His Glu Asp Leu Pro Val Cys Tyr Leu Leu  
35 40 45

Arg Val Leu Xaa  
50

<210> 146  
<211> 79  
<212> PRT  
<213> Homo sapiens

<220>  
<221> SITE  
<222> (79)  
<223> Xaa equals stop translation

<400> 146  
Met Ala Lys Arg Ser Ser Ser Leu Ser Ser Ser Lys Arg Leu Val Phe  
1 5 10 15

Phe Thr Ala Leu Ala Ser Trp Leu Trp Arg Val Pro Glu Ser Leu Gly  
20 25 30

Ser Pro Leu Asp Leu Leu Ser Asp Ala Lys Trp Val Cys Glu Ala Gly  
35 40 45

Ile Phe His Trp Ser Ser Ser Ser Leu Leu Asn Asn Arg Ala Asp Ala  
50 55 60

Phe Phe Leu Glu Ser Ser Glu Ala Phe Ala Phe Ser Ser Leu Xaa  
65 70 75

<210> 147  
<211> 48  
<212> PRT  
<213> Homo sapiens

<220>  
<221> SITE  
<222> (48)  
<223> Xaa equals stop translation

<400> 147  
Met Lys Met Asn Lys Leu Phe Trp Ile Arg Ile Leu Lys Leu Leu Leu  
1 5 10 15

Gln Ala Leu Ser Gln Cys Lys Leu Leu Ile Lys Gly Gln Val Ala Val  
20 25 30

Pro Lys Asp Leu Ile Met Asp Ser Glu Ile Ala Lys Val Thr Asn Xaa  
35 40 45

<210> 148  
<211> 54  
<212> PRT  
<213> Homo sapiens

<220>  
<221> SITE  
<222> (54)  
<223> Xaa equals stop translation

<400> 148  
Met Asn Leu Leu His Cys Leu Tyr Met Ile Asn Ile Ile Ile Tyr Ile  
1 5 10 15

Phe Cys Ile Lys Leu Ile Trp Leu His Leu Ser Cys Ile Leu Ser His  
20 25 30

Ile Ser Phe Ile Ser Ser Met Asp Met Ser Arg Ser Leu Tyr Trp Ser  
35 40 45

Pro Val Cys Ala Val Xaa  
50

117

<210> 149  
 <211> 311  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (311)  
 <223> Xaa equals stop translation

<400> 149

Met	Ala	Leu	Arg	Arg	Pro	Pro	Arg	Leu	Arg	Leu	Cys	Ala	Arg	Leu	Pro
1				5					10					15	
Asp	Phe	Phe	Leu	Leu	Leu	Leu	Phe	Arg	Gly	Cys	Leu	Ile	Gly	Ala	Val
			20					25					30		
Asn	Leu	Lys	Ser	Ser	Asn	Arg	Thr	Pro	Val	Val	Gln	Glu	Phe	Glu	Ser
		35					40					45			
Val	Glu	Leu	Ser	Cys	Ile	Ile	Thr	Asp	Ser	Gln	Thr	Ser	Asp	Pro	Arg
	50					55					60				
Ile	Glu	Trp	Lys	Lys	Ile	Gln	Asp	Glu	Gln	Thr	Thr	Tyr	Val	Phe	Phe
65					70					75				80	
Asp	Asn	Lys	Ile	Gln	Gly	Asp	Leu	Ala	Gly	Arg	Ala	Glu	Ile	Leu	Gly
				85					90					95	
Lys	Thr	Ser	Leu	Lys	Ile	Trp	Asn	Val	Thr	Arg	Arg	Asp	Ser	Ala	Leu
			100					105					110		
Tyr	Arg	Cys	Glu	Val	Val	Ala	Arg	Asn	Asp	Arg	Lys	Glu	Ile	Asp	Glu
		115					120					125			
Ile	Val	Ile	Glu	Leu	Thr	Val	Gln	Val	Lys	Pro	Val	Thr	Pro	Val	Cys
	130					135					140				
Arg	Val	Pro	Lys	Ala	Val	Pro	Val	Gly	Lys	Met	Ala	Thr	Leu	His	Cys
145					150					155					160
Gln	Glu	Ser	Glu	Gly	His	Pro	Arg	Pro	His	Tyr	Ser	Trp	Tyr	Arg	Asn
				165					170					175	
Asp	Val	Pro	Leu	Pro	Thr	Asp	Ser	Arg	Ala	Asn	Pro	Arg	Phe	Arg	Asn
			180					185					190		
Ser	Ser	Phe	His	Leu	Asn	Ser	Glu	Thr	Gly	Thr	Leu	Val	Phe	Thr	Ala
		195					200					205			



118

Val His Lys Asp Asp Ser Gly Gln Tyr Tyr Cys Ile Ala Ser Asn Asp  
 210 215 220  
 Ala Gly Ser Ala Arg Cys Glu Glu Gln Glu Met Glu Val Tyr Asp Leu  
 225 230 235 240  
 Asn Ile Gly Gly Ile Ile Gly Gly Val Leu Val Val Leu Ala Val Leu  
 245 250 255  
 Ala Leu Ile Thr Leu Gly Ile Cys Cys Ala Tyr Arg Arg Gly Tyr Phe  
 260 265 270  
 Ile Asn Asn Lys Gln Asp Gly Glu Ser Tyr Lys Asn Pro Gly Lys Pro  
 275 280 285  
 Asp Gly Val Asn Tyr Ile Arg Thr Asp Glu Glu Gly Asp Phe Arg His  
 290 295 300  
 Lys Ser Ser Phe Val Ile Xaa  
 305 310

<210> 150  
 <211> 311  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (311)  
 <223> Xaa equals stop translation

<400> 150  
 Met Ala Leu Arg Arg Pro Pro Arg Leu Arg Leu Cys Ala Arg Leu Pro  
 1 5 10 15  
 Asp Phe Phe Leu Leu Leu Leu Phe Arg Gly Cys Leu Ile Gly Ala Val  
 20 25 30  
 Asn Leu Lys Ser Ser Asn Arg Thr Pro Val Val Gln Glu Phe Glu Ser  
 35 40 45  
 Val Glu Leu Ser Cys Ile Ile Thr Asp Ser Gln Thr Ser Asp Pro Arg  
 50 55 60  
 Ile Glu Trp Lys Lys Ile Gln Asp Glu Gln Thr Thr Tyr Val Phe Phe  
 65 70 75 80  
 Asp Asn Lys Ile Gln Gly Asp Leu Ala Gly Arg Ala Glu Ile Leu Gly  
 85 90 95

119

Lys Thr Ser Leu Lys Ile Trp Asn Val Thr Arg Arg Asp Ser Ala Leu  
 100 105 110  
 Tyr Arg Cys Glu Val Val Ala Arg Asn Asp Arg Lys Glu Ile Asp Glu  
 115 120 125  
 Ile Val Ile Glu Leu Thr Val Gln Val Lys Pro Val Thr Pro Val Cys  
 130 135 140  
 Arg Val Pro Lys Ala Val Pro Val Gly Lys Met Ala Thr Leu His Cys  
 145 150 155 160  
 Gln Glu Ser Glu Gly His Pro Arg Pro His Tyr Ser Trp Tyr Arg Asn  
 165 170 175  
 Asp Val Pro Leu Pro Thr Asp Ser Arg Ala Asn Pro Arg Phe Arg Asn  
 180 185 190  
 Ser Ser Phe His Leu Asn Ser Glu Thr Gly Thr Leu Val Phe Thr Ala  
 195 200 205  
 Val His Lys Asp Asp Ser Gly Gln Tyr Tyr Cys Ile Ala Ser Asn Asp  
 210 215 220  
 Ala Gly Ser Ala Arg Cys Glu Glu Gln Glu Met Glu Val Tyr Asp Leu  
 225 230 235 240  
 Asn Ile Gly Gly Ile Ile Gly Gly Val Leu Val Val Leu Ala Val Leu  
 245 250 255  
 Ala Leu Ile Thr Leu Gly Ile Cys Cys Ala Tyr Arg Arg Gly Tyr Phe  
 260 265 270  
 Ile Asn Asn Lys Gln Asp Gly Glu Ser Tyr Lys Asn Pro Gly Lys Pro  
 275 280 285  
 Asp Gly Val Asn Tyr Ile Arg Thr Asp Glu Glu Gly Asp Phe Arg His  
 290 295 300  
 Lys Ser Ser Phe Val Ile Xaa  
 305 310

&lt;210&gt; 151

&lt;211&gt; 171

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

120

&lt;222&gt; (171)

&lt;223&gt; Xaa equals stop translation

&lt;400&gt; 151

Met Ile Leu Thr Met Leu Leu Met Leu Lys Leu Cys Thr Glu Val Arg  
 1 5 10 15

Val Ala Asn Glu Leu Asn Ala Arg Arg Arg Ser Phe Thr Asp Phe Asp  
 20 25 30

Pro His His Phe Trp Gln Trp Ser Ser Phe Ser Asp Tyr Val Gln Cys  
 35 40 45

Val Leu Ala Phe Thr Gly Val Ala Gly Tyr Ile Thr Tyr Leu Ser Ile  
 50 55 60

Asp Ser Ala Leu Phe Val Glu Thr Leu Gly Phe Leu Ala Val Leu Thr  
 65 70 75 80

Glu Ala Met Leu Gly Val Pro Gln Leu Tyr Arg Asn His Arg His Gln  
 85 90 95

Ser Thr Glu Gly Met Ser Ile Lys Met Val Leu Met Trp Thr Ser Gly  
 100 105 110

Asp Ala Phe Lys Thr Ala Tyr Phe Leu Leu Lys Gly Ala Pro Leu Gln  
 115 120 125

Phe Ser Val Cys Gly Leu Leu Gln Val Leu Val Asp Leu Ala Ile Leu  
 130 135 140

Gly Gln Ala Tyr Ala Phe Ala Arg His Pro Gln Lys Pro Ala Pro His  
 145 150 155 160

Ala Val His Pro Thr Gly Thr Lys Ala Leu Xaa  
 165 170

&lt;210&gt; 152

&lt;211&gt; 115

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (115)

&lt;223&gt; Xaa equals stop translation

&lt;400&gt; 152

Met Val Thr Arg Ala Gly Ala Gly Thr Ala Val Ala Gly Ala Val Val  
 1 5 10 15

121

Val Ala Leu Leu Ser Ala Ala Leu Ala Leu Tyr Gly Pro Pro Leu Asp  
                   20                  25                  30  
 Ala Val Leu Glu Arg Ala Phe Ser Leu Arg Lys Ala His Ser Ile Lys  
                   35                  40                  45  
 Asp Met Glu Asn Thr Leu Gln Leu Val Arg Asn Ile Ile Pro Pro Leu  
                   50                  55                  60  
 Ser Ser Thr Lys His Lys Gly Gln Asp Gly Arg Ile Gly Val Val Gly  
                   65                  70                  75                  80  
 Gly Cys Gln Glu Tyr Thr Gly Ala Pro Tyr Phe Ala Glu Ser Gln Leu  
                                   85                  90                  95  
 Ser Lys Trp Ala Gln Thr Cys Pro Thr Cys Ser Val Pro Val Arg Pro  
                   100                  105                  110  
 His Leu Xaa  
                   115

<210> 153  
 <211> 57  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (57)  
 <223> Xaa equals stop translation

<400> 153  
 Met Ala Arg Ala Cys Val Phe Gln Leu Ser Leu Trp Arg Lys Leu Pro  
       1                  5                  10                  15  
 Val Gly Ile Asn Leu Ser Pro Ala Ile Leu Ser Leu Ser Leu Gly Cys  
                   20                  25                  30  
 Leu Gly Leu Gly Phe Leu Leu Leu Leu Glu Arg Met Thr Thr Asp Ser  
                   35                  40                  45  
 Gly Ile Arg Gln Arg Arg Gln Thr Xaa  
                   50                  55

<210> 154  
 <211> 72  
 <212> PRT  
 <213> Homo sapiens

122

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (72)

&lt;223&gt; Xaa equals stop translation

&lt;400&gt; 154

Glu Ser Ala Pro Pro Trp Leu Pro Ile Cys Pro Thr Arg Ser Leu Gly  
 1 5 10 15

Leu Leu Val Gln Leu Leu Ala Leu Ala Gly Ser Cys Ser Ala Gly Pro  
 20 25 30

Arg Ala Leu Gly Gln Ala Ser Gly Val Val Arg Thr Thr Lys Pro Leu  
 35 40 45

Leu Ser Pro Ser Thr Pro Leu Asp Leu Gly Pro Pro Glu Pro Pro Ala  
 50 55 60

Gly Trp Ala Tyr Thr Ser Ser Xaa  
 65 70

&lt;210&gt; 155

&lt;211&gt; 155

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 155

Met Glu Asn Phe Ile Lys Val Gln Leu Arg Asp Gly Asp Ser Asn Cys  
 1 5 10 15

Glu Trp Ser Val Leu Tyr Val Ile Ile Ala Thr Phe Val Ile Val Val  
 20 25 30

Ala Leu Gly Ile Leu Ser Trp Thr Val Ile Cys Cys Cys Lys Arg Gln  
 35 40 45

Lys Gly Lys Pro Lys Arg Lys Ser Lys Tyr Lys Ile Leu Asp Ala Thr  
 50 55 60

Asp Gln Glu Ser Leu Glu Leu Lys Pro Thr Ser Arg Ala Gly Lys Glu  
 65 70 75 80

Lys Arg Met Ser Leu Ser Gly Leu Asn Gln Ser Ser Trp Ile Leu Glu  
 85 90 95

Met Lys Asn Gln Gln Glu Thr Pro Gly Ile Lys Gln Lys Gly Leu Leu  
 100 105 110

Leu Ser Ser Ser Leu Met His Ser Glu Ser Glu Leu Asp Ser Asp Asp

123

115 120 125  
Ala Ile Phe Thr Trp Pro Asp Arg Glu Lys Gly Lys Leu Leu His Gly  
130 135 140  
Gln Asn Gly Ser Val Pro Asn Gly Arg Pro Leu  
145 150 155  
  
<210> 156  
<211> 103  
<212> PRT  
<213> Homo sapiens  
  
<220>  
<221> SITE  
<222> (103)  
<223> Xaa equals stop translation  
  
<400> 156  
Met Asn Pro Ala Val Arg Gln Arg Cys Leu Leu Phe Cys Phe Gln Gln.  
1 5 10 15  
Lys Leu Ile Leu Ser His Phe Phe Leu Leu Gln Val Pro Gln Trp Cys  
20 25 30  
Ala Glu Tyr Cys Leu Ser Ile His Tyr Gln His Gly Gly Val Ile Cys  
35 40 45  
Thr Gln Val His Lys Gln Thr Val Val Gln Leu Ala Leu Arg Val Ala  
50 55 60  
Asp Glu Met Asp Val Asn Ile Gly His Glu Val Gly Tyr Val Ile Pro  
65 70 75 80  
Phe Glu Asn Cys Cys Thr Asn Glu Thr Ile Leu Arg Leu Val Cys Gly  
85 90 95  
Val Gln Ser Ala Pro Cys Xaa  
100

<210> 157  
<211> 59  
<212> PRT  
<213> Homo sapiens  
  
<220>  
<221> SITE  
<222> (59)  
<223> Xaa equals stop translation

124

&lt;400&gt; 157

Met Val Ser Phe Gly Phe Trp Phe Leu Cys Leu Phe Phe Gly Val Trp  
1 5 10 15

Lys Asn Met His Phe Tyr Arg Ala Arg Lys Leu Val Ser Arg Lys Gly  
20 25 30

Ser Pro Glu Lys Ala Ala Asp Gly Pro Cys Pro Cys Trp Val Phe Leu  
35 40 45

Phe Phe Gly Thr Val Arg Gly Asn Gly Phe Xaa  
50 55

&lt;210&gt; 158

&lt;211&gt; 46

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (46)

&lt;223&gt; Xaa equals stop translation

&lt;400&gt; 158

Met Val Gln Trp Lys Asn Trp Pro Glu Ser Leu Glu Val Trp Val Leu  
1 5 10 15

Val Leu Ala Val Pro Leu Thr His Cys Asp Leu Gly Ile Leu Cys Cys  
20 25 30

Glu Asp Ile Ser Gln Val Leu His Val Ser Gln Gln Ile Xaa  
35 40 45

&lt;210&gt; 159

&lt;211&gt; 99

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (99)

&lt;223&gt; Xaa equals stop translation

&lt;400&gt; 159

Met Val His Ile Asn Arg Ala Leu Lys Leu Ile Ile Arg Leu Phe Leu  
1 5 10 15

Val Glu Asp Leu Val Asp Ser Leu Lys Leu Ala Val Phe Met Trp Leu

125

20                      25                      30  
 Met Thr Tyr Val Gly Ala Val Phe Asn Gly Ile Thr Leu Leu Ile Leu  
           35                      40                      45  
 Ala Glu Leu Leu Ile Phe Ser Val Pro Ile Val Tyr Glu Lys Tyr Lys  
           50                      55                      60  
 Thr Gln Ile Asp His Tyr Val Gly Ile Ala Arg Asp Gln Thr Lys Ser  
           65                      70                      75                      80  
 Ile Val Glu Lys Ile Gln Ala Lys Leu Pro Gly Ile Ala Lys Lys Lys  
                                  85                      90                      95  
 Ala Glu Xaa

<210> 160  
 <211> 392  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (251)  
 <223> Xaa equals any of the naturally occurring L-amino acids

<400> 160  
 Met Ala Pro Trp Pro Pro Lys Gly Leu Val Pro Ala Val Leu Trp Gly  
           1                      5                      10                      15  
 Leu Ser Leu Phe Leu Asn Leu Pro Gly Pro Ile Trp Leu Gln Pro Ser  
                                  20                      25                      30  
 Pro Pro Pro Gln Ser Ser Pro Pro Pro Gln Pro His Pro Cys His Thr  
                                  35                      40                      45  
 Cys Arg Gly Leu Val Asp Ser Phe Asn Lys Gly Leu Glu Arg Thr Ile  
           50                      55                      60  
 Arg Asp Asn Phe Gly Gly Gly Asn Thr Ala Trp Glu Glu Glu Asn Leu  
           65                      70                      75                      80  
 Ser Lys Tyr Lys Asp Ser Glu Thr Arg Leu Val Glu Val Leu Glu Gly  
                                  85                      90                      95  
 Val Cys Ser Lys Ser Asp Phe Glu Cys His Arg Leu Leu Glu Leu Ser  
                                  100                      105                      110  
 Glu Glu Leu Val Glu Ser Trp Trp Phe His Lys Gln Gln Glu Ala Pro



126

115	120	125
Asp Leu Phe Gln Trp Leu Cys Ser Asp Ser Leu Lys Leu Cys Cys Pro		
130	135	140
Ala Gly Thr Phe Gly Pro Ser Cys Leu Pro Cys Pro Gly Gly Thr Glu		
145	150	155 160
Arg Pro Cys Gly Gly Tyr Gly Gln Cys Glu Gly Glu Gly Thr Arg Gly		
	165	170 175
Gly Ser Gly His Cys Asp Cys Gln Ala Gly Tyr Gly Gly Glu Ala Cys		
	180	185 190
Gly Gln Cys Gly Leu Gly Tyr Phe Glu Ala Glu Arg Asn Ala Ser His		
	195	200 205
Leu Val Cys Ser Ala Cys Phe Gly Pro Cys Ala Arg Cys Ser Gly Pro		
	210	215 220
Glu Glu Ser Asn Cys Leu Gln Cys Lys Lys Gly Trp Ala Leu His His		
	225	230 235 240
Leu Lys Cys Val Asp Cys Ala Lys Ala Cys Xaa Gly Cys Met Gly Ala		
	245	250 255
Gly Pro Gly Arg Cys Lys Lys Cys Ser Pro Gly Tyr Gln Gln Val Gly		
	260	265 270
Ser Lys Cys Leu Asp Val Asp Glu Cys Glu Thr Glu Val Cys Pro Gly		
	275	280 285
Glu Asn Lys Gln Cys Glu Asn Thr Glu Gly Gly Tyr Arg Cys Ile Cys		
	290	295 300
Ala Glu Gly Tyr Lys Gln Met Glu Gly Ile Cys Val Lys Glu Gln Ile		
	305	310 315 320
Pro Glu Ser Ala Gly Phe Phe Ser Glu Met Thr Glu Asp Glu Leu Val		
	325	330 335
Val Leu Gln Gln Met Phe Phe Gly Ile Ile Ile Cys Ala Leu Ala Thr		
	340	345 350
Leu Ala Ala Lys Gly Asp Leu Val Phe Thr Ala Ile Phe Ile Gly Ala		
	355	360 365
Val Ala Ala Met Thr Gly Tyr Trp Leu Ser Glu Arg Ser Asp Arg Val		
	370	375 380
Leu Glu Gly Phe Ile Lys Gly Arg		

127

385

390

&lt;210&gt; 161

&lt;211&gt; 434

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 161

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Met Ala Pro Glu Gly Leu Val Pro Ala Val Leu Trp Gly Leu Ser Leu
  1             5             10             15

Phe Leu Asn Leu Pro Gly Pro Ile Trp Leu Gln Pro Ser Pro Pro Pro
      20             25             30

Gln Ser Ser Pro Pro Pro Gln Pro His Pro Cys His Thr Cys Arg Gly
      35             40             45

Leu Val Asp Ser Phe Asn Lys Gly Leu Glu Arg Thr Ile Arg Asp Asn
      50             55             60

Phe Gly Gly Gly Asn Thr Ala Trp Glu Glu Glu Asn Leu Ser Lys Tyr
      65             70             75             80

Lys Asp Ser Glu Thr Arg Leu Val Glu Val Leu Glu Gly Val Cys Ser
      85             90             95

Lys Ser Asp Phe Glu Cys His Arg Leu Leu Glu Leu Ser Glu Glu Leu
      100            105            110

Val Glu Ser Trp Trp Phe His Lys Gln Gln Glu Ala Pro Asp Leu Phe
      115            120            125

Gln Trp Leu Cys Ser Asp Ser Leu Lys Leu Cys Cys Pro Ala Gly Thr
      130            135            140

Phe Gly Pro Ser Cys Leu Pro Cys Pro Gly Gly Thr Glu Arg Pro Cys
      145            150            155            160

Gly Gly Tyr Gly Gln Cys Glu Gly Glu Gly Thr Arg Gly Gly Ser Gly
      165            170            175

His Cys Asp Cys Gln Ala Gly Tyr Gly Gly Glu Ala Cys Gly Gln Cys
      180            185            190

Gly Leu Gly Tyr Phe Glu Ala Glu Arg Asn Ala Ser His Leu Val Cys
      195            200            205

Ser Ala Cys Phe Gly Pro Cys Ala Arg Cys Ser Gly Pro Glu Glu Ser
      210            215            220

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128

Asn Cys Leu Gln Cys Lys Lys Gly Trp Ala Leu His His Leu Lys Cys  
 225 230 235 240  
 Val Asp Ile Asp Glu Cys Gly Thr Glu Gly Ala Asn Cys Gly Ala Asp  
 245 250 255  
 Gln Phe Cys Val Asn Thr Glu Gly Ser Tyr Glu Cys Arg Asp Cys Ala  
 260 265 270  
 Lys Ala Cys Leu Gly Cys Met Gly Ala Gly Pro Gly Arg Cys Lys Lys  
 275 280 285  
 Cys Ser Pro Gly Tyr Gln Gln Val Gly Ser Lys Cys Leu Asp Val Asp  
 290 295 300  
 Glu Cys Glu Thr Glu Val Cys Pro Gly Glu Asn Lys Gln Cys Glu Asn  
 305 310 315 320  
 Thr Glu Gly Gly Tyr Arg Cys Ile Cys Ala Glu Gly Tyr Lys Gln Met  
 325 330 335  
 Glu Gly Ile Cys Val Lys Glu Gln Ile Pro Gly Ala Phe Pro Ile Leu  
 340 345 350  
 Thr Asp Leu Thr Pro Glu Thr Thr Arg Arg Trp Lys Leu Gly Ser His  
 355 360 365  
 Pro His Ser Thr Tyr Val Lys Met Lys Met Gln Arg Asp Glu Ala Thr  
 370 375 380  
 Phe Pro Gly Leu Tyr Gly Lys Gln Val Ala Lys Leu Gly Ser Gln Ser  
 385 390 395 400  
 Arg Gln Ser Asp Arg Gly Thr Arg Leu Ile His Val Ile Asn Ala Leu  
 405 410 415  
 Pro Pro Thr Cys Pro Pro Gln Lys Lys Lys Lys Lys Lys Lys Lys Gly  
 420 425 430  
 Gly Arg

&lt;210&gt; 162

&lt;211&gt; 151

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (151)

129

&lt;223&gt; Xaa equals stop translation

&lt;400&gt; 162

Met Val Met Ile Leu Phe Val Ala Phe Ile Thr Cys Trp Glu Glu Val  
 1 5 10 15

Thr Thr Leu Val Gln Ala Ile Arg Ile Thr Ser Tyr Met Asn Glu Thr  
 20 25 30

Ile Leu Tyr Phe Pro Phe Ser Ser His Ser Ser Tyr Thr Val Arg Ser  
 35 40 45

Lys Lys Ile Phe Leu Ser Lys Leu Ile Val Cys Phe Leu Ser Thr Trp  
 50 55 60

Leu Pro Phe Val Leu Leu Gln Val Ile Ile Val Leu Leu Lys Val Gln  
 65 70 75 80

Ile Pro Ala Tyr Ile Glu Met Asn Ile Pro Trp Leu Tyr Phe Val Asn  
 85 90 95

Ser Phe Leu Ile Ala Thr Val Tyr Trp Phe Asn Cys His Lys Leu Asn  
 100 105 110

Leu Lys Asp Ile Gly Leu Pro Leu Asp Pro Phe Val Asn Trp Lys Cys  
 115 120 125

Cys Phe Ile Pro Leu Thr Ile Pro Asn Leu Glu Gln Ile Glu Lys Pro  
 130 135 140

Ile Ser Ile Met Ile Cys Xaa  
 145 150

&lt;210&gt; 163

&lt;211&gt; 110

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 163

His Ala Ser Gly Trp Arg Thr Pro Arg Asp Pro Glu Arg Pro Pro Arg  
 1 5 10 15

His Ile Gln Thr Ser Ala Ala Pro Ala Pro Ser Gln Pro Ser Trp Asp  
 20 25 30

Ser Arg Ala His Pro Thr Gln Arg Arg Asp Pro Gly Pro Pro Gly Pro  
 35 40 45

Ser Ala Asp Ser Thr Ala His Phe Pro Gly Pro Pro His Thr Ser Gln  
 50 55 60

130

Pro Ser Gly Arg Ser Leu Pro Thr Arg Cys Arg Val Pro Pro Ala Leu  
65 70 75 80

Ser Arg Pro Gly Ser Pro Pro Pro Gly Pro Arg Gly Gly Pro Ser Gln  
85 90 95

Ala Pro Phe Glu Pro Arg Arg Arg Pro Gly Leu Gly Arg Thr  
100 105 110

&lt;210&gt; 164

&lt;211&gt; 56

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 164

His Ala Ser Gly Trp Arg Thr Pro Arg Asp Pro Glu Arg Pro Pro Arg  
1 5 10 15

His Ile Gln Thr Ser Ala Ala Pro Ala Pro Ser Gln Pro Ser Trp Asp  
20 25 30

Ser Arg Ala His Pro Thr Gln Arg Arg Asp Pro Gly Pro Pro Gly Pro  
35 40 45

Ser Ala Asp Ser Thr Ala His Phe  
50 55

&lt;210&gt; 165

&lt;211&gt; 54

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 165

Pro Gly Pro Pro His Thr Ser Gln Pro Ser Gly Arg Ser Leu Pro Thr  
1 5 10 15

Arg Cys Arg Val Pro Pro Ala Leu Ser Arg Pro Gly Ser Pro Pro Pro  
20 25 30

Gly Pro Arg Gly Gly Pro Ser Gln Ala Pro Phe Glu Pro Arg Arg Arg  
35 40 45

Pro Gly Leu Gly Arg Thr  
50

&lt;210&gt; 166

&lt;211&gt; 723

131

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 166

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His Ala Ser Ala Ser Pro Gly Arg Val Asp Ala Asp Ser Asn Ala Val
  1              5              10              15

Ala Ser Gly Pro Arg Thr Pro Ser Gly Pro Thr Arg Gln Glu Arg Leu
      20              25              30

Arg Pro Arg Pro Ala Pro Pro Gly Ser Leu Arg Arg Arg Arg Leu Pro
      35              40              45

Gly Gln Lys Met Cys Ser Arg Val Pro Leu Leu Leu Pro Leu Leu Leu
      50              55              60

Leu Leu Ala Leu Gly Pro Gly Val Gln Gly Cys Pro Ser Gly Cys Gln
      65              70              75              80

Cys Ser Gln Pro Gln Thr Val Phe Cys Thr Ala Arg Gln Gly Thr Thr
      85              90              95

Val Pro Arg Asp Val Pro Pro Asp Thr Val Gly Leu Tyr Val Phe Glu
      100             105             110

Asn Gly Ile Thr Met Leu Asp Ala Gly Ser Phe Ala Gly Leu Pro Gly
      115             120             125

Leu Gln Leu Leu Asp Leu Ser Gln Asn Gln Ile Ala Ser Leu Pro Ser
      130             135             140

Gly Val Phe Gln Pro Leu Ala Asn Leu Ser Asn Leu Asp Leu Thr Ala
      145             150             155             160

Asn Arg Leu His Glu Ile Thr Asn Glu Thr Phe Arg Gly Leu Arg Arg
      165             170             175

Leu Glu Arg Leu Tyr Leu Gly Lys Asn Arg Ile Arg His Ile Gln Pro
      180             185             190

Gly Ala Phe Asp Thr Leu Asp Arg Leu Leu Glu Leu Lys Leu Gln Asp
      195             200             205

Asn Glu Leu Arg Ala Leu Pro Pro Leu Arg Leu Pro Arg Leu Leu Leu
      210             215             220

Leu Asp Leu Ser His Asn Ser Leu Leu Ala Leu Glu Pro Gly Ile Leu
      225             230             235             240

Asp Thr Ala Asn Val Glu Ala Leu Arg Leu Ala Gly Leu Gly Leu Gln
      245             250             255

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Gln Leu Asp Glu Gly Leu Phe Ser Arg Leu Arg Asn Leu His Asp Leu  
 260 265 270  
 Asp Val Ser Asp Asn Gln Leu Glu Arg Val Pro Pro Val Ile Arg Gly  
 275 280 285  
 Leu Arg Gly Leu Thr Arg Leu Arg Leu Ala Gly Asn Thr Arg Ile Ala  
 290 295 300  
 Gln Leu Arg Pro Glu Asp Leu Ala Gly Leu Ala Ala Leu Gln Glu Leu  
 305 310 315 320  
 Asp Val Ser Asn Leu Ser Leu Gln Ala Leu Pro Gly Asp Leu Ser Gly  
 325 330 335  
 Leu Phe Pro Arg Leu Arg Leu Leu Ala Ala Ala Arg Asn Pro Phe Asn  
 340 345 350  
 Cys Val Cys Pro Leu Ser Trp Phe Gly Pro Trp Val Arg Glu Ser His  
 355 360 365  
 Val Thr Leu Ala Ser Pro Glu Glu Thr Arg Cys His Phe Pro Pro Lys  
 370 375 380  
 Asn Ala Gly Arg Leu Leu Leu Glu Leu Asp Tyr Ala Asp Phe Gly Cys  
 385 390 395 400  
 Pro Ala Thr Thr Thr Thr Ala Thr Val Pro Thr Thr Arg Pro Val Val  
 405 410 415  
 Arg Glu Pro Thr Ala Leu Ser Ser Ser Leu Ala Pro Thr Trp Leu Ser  
 420 425 430  
 Pro Thr Ala Pro Ala Thr Glu Ala Pro Ser Pro Pro Ser Thr Ala Pro  
 435 440 445  
 Pro Thr Val Gly Pro Val Pro Gln Pro Gln Asp Cys Pro Pro Ser Thr  
 450 455 460  
 Cys Leu Asn Gly Gly Thr Cys His Leu Gly Thr Arg His His Leu Ala  
 465 470 475 480  
 Cys Leu Cys Pro Glu Gly Phe Thr Gly Leu Tyr Cys Glu Ser Gln Met  
 485 490 495  
 Gly Gln Gly Thr Arg Pro Ser Pro Thr Pro Val Thr Pro Arg Pro Pro  
 500 505 510  
 Arg Ser Leu Thr Leu Gly Ile Glu Pro Val Ser Pro Thr Ser Leu Arg  
 515 520 525

Val Gly Leu Gln Arg Tyr Leu Gln Gly Ser Ser Val Gln Leu Arg Ser  
 530 535 540  
 Leu Arg Leu Thr Tyr Arg Asn Leu Ser Gly Pro Asp Lys Arg Leu Val  
 545 550 555 560  
 Thr Leu Arg Leu Pro Ala Ser Leu Ala Glu Tyr Thr Val Thr Gln Leu  
 565 570 575  
 Arg Pro Asn Ala Thr Tyr Ser Val Cys Val Met Pro Leu Gly Pro Gly  
 580 585 590  
 Arg Val Pro Glu Gly Glu Glu Ala Cys Gly Glu Ala His Thr Pro Pro  
 595 600 605  
 Ala Val His Ser Asn His Ala Pro Val Thr Gln Ala Arg Glu Gly Asn  
 610 615 620  
 Leu Pro Leu Leu Ile Ala Pro Ala Leu Ala Ala Val Leu Leu Ala Ala  
 625 630 635 640  
 Leu Ala Ala Val Gly Ala Ala Tyr Cys Val Arg Arg Gly Arg Ala Met  
 645 650 655  
 Ala Ala Ala Ala Gln Asp Lys Gly Gln Val Gly Pro Gly Ala Gly Pro.  
 660 665 670  
 Leu Glu Leu Glu Gly Val Lys Val Pro Leu Glu Pro Gly Pro Lys Ala  
 675 680 685  
 Thr Glu Ala Val Glu Arg Pro Cys Pro Ala Gly Leu Ser Val Lys Cys  
 690 695 700  
 His Ser Trp Ala Ser Lys Ala Trp Pro Gln Ser Pro Leu His Ala Lys  
 705 710 715 720  
 Pro Tyr Ile

&lt;210&gt; 167

&lt;211&gt; 51

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 167

His Ala Ser Gly Arg Leu Gln Thr Gln Arg Glu Gly Gly Gln Gly Val  
 1 5 10 15

Gly Arg Arg Arg Thr Glu Glu Gly Thr Glu Thr Gln Ser Lys Gly Gly



134

20 25 30  
 Lys Glu Glu Thr Leu Val Gly Gly Arg His Ser Gly Glu Arg Gly Gly  
 35 40 45  
 Trp Ala Glu  
 50  
 <210> 168  
 <211> 59  
 <212> PRT  
 <213> Homo sapiens  
 <400> 168  
 Pro Arg Val Arg Ala Glu Ser Glu Gly Thr Tyr Asp Thr Tyr Gln His  
 1 5 10 15  
 Val Pro Val Glu Ser Phe Ala Glu Val Leu Leu Arg Thr Gly Lys Leu  
 20 25 30  
 Ala Glu Ala Lys Asn Lys Gly Glu Val Phe Pro Thr Thr Glu Val Leu  
 35 40 45  
 Leu Gln Leu Ala Ser Glu Ala Leu Pro Asn Asp  
 50 55  
 <210> 169  
 <211> 454  
 <212> PRT  
 <213> Homo sapiens  
 <400> 169  
 Leu Glu Arg Leu Leu Arg Gln Ser Asn Ile Leu Leu Arg Ser Pro Arg  
 1 5 10 15  
 Lys Arg Asn Ser Glu Asp Glu Ala Gln Glu Ala Lys Asp Ser Lys Val  
 20 25 30  
 Thr Tyr Ala Asp Thr Leu Asn His Leu Glu Lys Ser Leu Ala His Leu  
 35 40 45  
 Glu Thr Leu Ser His Ser Phe Ile Leu Ser Leu Lys Asn Ser Glu Gln  
 50 55 60  
 Glu Thr Leu Gln Lys Tyr Ser His Leu Tyr Asp Leu Ser Arg Ser Glu  
 65 70 75 80  
 Lys Glu Lys Leu His Asp Glu Ala Val Ala Ile Cys Leu Asp Gly Gln  
 85 90 95

135

Pro Leu Ala Met Ile Gln Gln Leu Leu Glu Val Ala Val Gly Pro Leu  
 100 105 110

Asp Ile Ser Pro Lys Asp Ile Val Gln Ser Ala Ile Met Lys Ile Ile  
 115 120 125

Ser Ala Leu Ser Gly Gly Ser Ala Asp Leu Gly Gly Pro Arg Asp Pro  
 130 135 140

Leu Lys Val Leu Glu Gly Val Val Ala Ala Val His Ala Ser Val Asp  
 145 150 155 160

Lys Gly Glu Glu Leu Val Ser Pro Glu Asp Leu Leu Glu Trp Leu Arg  
 165 170 175

Pro Phe Cys Ala Asp Asp Ala Trp Pro Val Arg Pro Arg Ile His Val  
 180 185 190

Leu Gln Ile Leu Gly Gln Ser Phe His Leu Thr Glu Glu Asp Ser Lys  
 195 200 205

Leu Leu Val Phe Phe Arg Thr Glu Ala Ile Leu Lys Ala Ser Trp Pro  
 210 215 220

Gln Arg Gln Val Asp Ile Ala Asp Ile Glu Asn Glu Glu Asn Arg Tyr  
 225 230 235 240

Cys Leu Phe Met Glu Leu Leu Glu Ser Ser His His Glu Ala Glu Phe  
 245 250 255

Gln His Leu Val Leu Leu Leu Gln Ala Trp Pro Pro Met Lys Ser Glu  
 260 265 270

Tyr Val Ile Thr Asn Asn Pro Trp Val Arg Leu Ala Thr Val Met Leu  
 275 280 285

Thr Arg Cys Thr Met Glu Asn Lys Glu Gly Leu Gly Asn Glu Val Leu  
 290 295 300

Lys Met Cys Arg Ser Leu Tyr Asn Thr Lys Gln Met Leu Pro Ala Glu  
 305 310 315 320

Gly Val Lys Glu Leu Cys Leu Leu Leu Leu Asn Gln Ser Leu Leu Leu  
 325 330 335

Pro Ser Leu Lys Leu Leu Leu Glu Ser Arg Asp Glu His Leu His Glu  
 340 345 350

Met Ala Leu Glu Gln Ile Thr Ala Val Thr Thr Val Asn Asp Ser Asn  
 355 360 365

136

Cys Asp Gln Glu Leu Leu Ser Leu Leu Leu Asp Ala Lys Leu Leu Val  
 370 375 380

Lys Cys Val Ser Thr Pro Phe Tyr Pro Arg Ile Val Asp His Leu Leu  
 385 390 395 400

Ala Ser Leu Gln Gln Gly Arg Trp Asp Ala Glu Glu Leu Gly Arg His  
 405 410 415

Leu Arg Glu Ala Gly His Glu Ala Glu Ala Gly Ser Leu Leu Leu Ala  
 420 425 430

Val Arg Gly Thr His Gln Ala Phe Arg Thr Phe Ser Thr Ala Leu Arg  
 435 440 445

Ala Ala Gln His Trp Val  
 450

&lt;210&gt; 170

&lt;211&gt; 36

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 170

Leu Glu Arg Leu Leu Arg Gln Ser Asn Ile Leu Leu Arg Ser Pro Arg  
 1 5 10 15

Lys Arg Asn Ser Glu Asp Glu Ala Gln Glu Ala Lys Asp Ser Lys Val  
 20 25 30

Thr Tyr Ala Asp  
 35

&lt;210&gt; 171

&lt;211&gt; 35

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 171

Thr Leu Asn His Leu Glu Lys Ser Leu Ala His Leu Glu Thr Leu Ser  
 1 5 10 15

His Ser Phe Ile Leu Ser Leu Lys Asn Ser Glu Gln Glu Thr Leu Gln  
 20 25 30

Lys Tyr Ser  
 35

137

<210> 172  
<211> 36  
<212> PRT  
<213> Homo sapiens

<400> 172  
His Leu Tyr Asp Leu Ser Arg Ser Glu Lys Glu Lys Leu His Asp Glu  
1 5 10 15  
Ala Val Ala Ile Cys Leu Asp Gly Gln Pro Leu Ala Met Ile Gln Gln  
20 25 30  
Leu Leu Glu Val  
35

<210> 173  
<211> 35  
<212> PRT  
<213> Homo sapiens

<400> 173  
Ala Val Gly Pro Leu Asp Ile Ser Pro Lys Asp Ile Val Gln Ser Ala  
1 5 10 15  
Ile Met Lys Ile Ile Ser Ala Leu Ser Gly Gly Ser Ala Asp Leu Gly  
20 25 30  
Gly Pro Arg  
35

<210> 174  
<211> 36  
<212> PRT  
<213> Homo sapiens

<400> 174  
Asp Pro Leu Lys Val Leu Glu Gly Val Val Ala Ala Val His Ala Ser  
1 5 10 15  
Val Asp Lys Gly Glu Glu Leu Val Ser Pro Glu Asp Leu Leu Glu Trp  
20 25 30  
Leu Arg Pro Phe  
35

<210> 175  
<211> 35

138

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 175

Cys Ala Asp Asp Ala Trp Pro Val Arg Pro Arg Ile His Val Leu Gln  
1 5 10 15

Ile Leu Gly Gln Ser Phe His Leu Thr Glu Glu Asp Ser Lys Leu Leu  
20 25 30

Val Phe Phe  
35

&lt;210&gt; 176

&lt;211&gt; 37

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 176

Arg Thr Glu Ala Ile Leu Lys Ala Ser Trp Pro Gln Arg Gln Val Asp  
1 5 10 15

Ile Ala Asp Ile Glu Asn Glu Glu Asn Arg Tyr Cys Leu Phe Met Glu  
20 25 30

Leu Leu Glu Ser Ser  
35

&lt;210&gt; 177

&lt;211&gt; 34

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 177

His His Glu Ala Glu Phe Gln His Leu Val Leu Leu Leu Gln Ala Trp  
1 5 10 15

Pro Pro Met Lys Ser Glu Tyr Val Ile Thr Asn Asn Pro Trp Val Arg  
20 25 30

Leu Ala

&lt;210&gt; 178

&lt;211&gt; 36

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

139

&lt;400&gt; 178

Thr Val Met Leu Thr Arg Cys Thr Met Glu Asn Lys Glu Gly Leu Gly  
1 5 10 15

Asn Glu Val Leu Lys Met Cys Arg Ser Leu Tyr Asn Thr Lys Gln Met  
20 25 30

Leu Pro Ala Glu  
35

&lt;210&gt; 179

&lt;211&gt; 35

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 179

Gly Val Lys Glu Leu Cys Leu Leu Leu Asn Gln Ser Leu Leu Leu  
1 5 10 15

Pro Ser Leu Lys Leu Leu Leu Glu Ser Arg Asp Glu His Leu His Glu  
20 25 30

Met Ala Leu  
35

&lt;210&gt; 180

&lt;211&gt; 36

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 180

Glu Gln Ile Thr Ala Val Thr Thr Val Asn Asp Ser Asn Cys Asp Gln  
1 5 10 15

Glu Leu Leu Ser Leu Leu Leu Asp Ala Lys Leu Leu Val Lys Cys Val  
20 25 30

Ser Thr Pro Phe  
35

&lt;210&gt; 181

&lt;211&gt; 35

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 181

Tyr Pro Arg Ile Val Asp His Leu Leu Ala Ser Leu Gln Gln Gly Arg  
1 5 10 15

140

Trp Asp Ala Glu Glu Leu Gly Arg His Leu Arg Glu Ala Gly His Glu  
                   20                  25                  30

Ala Glu Ala  
           35

<210> 182  
 <211> 28  
 <212> PRT  
 <213> Homo sapiens

<400> 182  
 Gly Ser Leu Leu Leu Ala Val Arg Gly Thr His Gln Ala Phe Arg Thr  
   1                  5                  10                  15

Phe Ser Thr Ala Leu Arg Ala Ala Gln His Trp Val  
                   20                  25

<210> 183  
 <211> 323  
 <212> PRT  
 <213> Homo sapiens

<400> 183  
 Pro Ser Ser Tyr Thr Ala Thr Met Asn Val Ser Trp Ile Ser Leu Arg  
   1                  5                  10                  15

Arg Arg Ser Phe Arg Ala Phe Gly Arg Val Trp Thr Cys Ser Gly Leu  
                   20                  25                  30

Leu Gln Met Thr Ser Ile Lys Gly Lys Leu Ser Leu Val Trp Gln Arg  
                   35                  40                  45

Leu Asp Gly His Phe Cys Arg Thr Leu Glu Glu Ser Val Tyr Ser Ile  
                   50                  55                  60

Ala Ile Ser Leu Ala Gln Arg Tyr Ser Val Ser Arg Trp Glu Val Phe  
   65                  70                  75                  80

Met Thr His Leu Glu Phe Leu Phe Thr Asp Ser Gly Leu Ser Thr Leu  
                   85                  90                  95

Glu Ile Glu Asn Arg Ala Gln Asp Leu His Leu Phe Glu Thr Leu Lys  
                   100                  105                  110

Thr Asp Pro Glu Ala Phe His Gln His Met Val Lys Tyr Ile Tyr Pro  
                   115                  120                  125

141

Thr Ile Gly Gly Phe Asp His Glu Arg Leu Gln Tyr Tyr Phe Thr Leu  
 130 135 140  
 Leu Glu Asn Cys Gly Cys Ala Asp Leu Gly Asn Cys Ala Ile Lys Pro  
 145 150 155 160  
 Glu Thr His Ile Arg Leu Leu Lys Lys Phe Lys Val Val Ala Ser Gly  
 165 170 175  
 Leu Asn Tyr Lys Lys Leu Thr Asp Glu Asn Met Ser Pro Leu Glu Ala  
 180 185 190  
 Leu Glu Pro Val Leu Ser Ser Gln Asn Ile Leu Ser Ile Ser Lys Leu  
 195 200 205  
 Val Pro Lys Ile Pro Glu Lys Asp Gly Gln Met Leu Ser Pro Ser Ser  
 210 215 220  
 Leu Tyr Thr Ile Trp Leu Gln Lys Leu Phe Trp Thr Gly Asp Pro His  
 225 230 235 240  
 Leu Ile Lys Gln Val Pro Gly Ser Ser Pro Glu Trp Leu His Ala Tyr  
 245 250 255  
 Asp Val Cys Met Lys Tyr Phe Asp Arg Leu His Pro Gly Asp Leu Ile  
 260 265 270  
 Thr Val Val Asp Ala Val Thr Phe Ser Pro Lys Ala Val Thr Lys Leu  
 275 280 285  
 Ser Val Glu Ala Arg Lys Glu Met Thr Arg Lys Ala Ile Lys Thr Val  
 290 295 300  
 Lys His Phe Ile Glu Lys Pro Lys Glu Lys Lys Leu Arg Arg Arg Ser  
 305 310 315 320  
 Ser Arg Ser

&lt;210&gt; 184

&lt;211&gt; 38

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 184

Pro Ser Ser Tyr Thr Ala Thr Met Asn Val Ser Trp Ile Ser Leu Arg  
 1 5 10 15

Arg Arg Ser Phe Arg Ala Phe Gly Arg Val Trp Thr Cys Ser Gly Leu  
 20 25 30



142

Leu Gln Met Thr Ser Ile  
35

<210> 185  
<211> 33  
<212> PRT  
<213> Homo sapiens

<400> 185  
Lys Gly Lys Leu Ser Leu Val Trp Gln Arg Leu Asp Gly His Phe Cys  
1 5 10 15  
Arg Thr Leu Glu Glu Ser Val Tyr Ser Ile Ala Ile Ser Leu Ala Gln  
20 25 30

Arg

<210> 186  
<211> 35  
<212> PRT  
<213> Homo sapiens

<400> 186  
Tyr Ser Val Ser Arg Trp Glu Val Phe Met Thr His Leu Glu Phe Leu  
1 5 10 15  
Phe Thr Asp Ser Gly Leu Ser Thr Leu Glu Ile Glu Asn Arg Ala Gln  
20 25 30

Asp Leu His  
35

<210> 187  
<211> 36  
<212> PRT  
<213> Homo sapiens

<400> 187  
Leu Phe Glu Thr Leu Lys Thr Asp Pro Glu Ala Phe His Gln His Met  
1 5 10 15  
Val Lys Tyr Ile Tyr Pro Thr Ile Gly Gly Phe Asp His Glu Arg Leu  
20 25 30

Gln Tyr Tyr Phe  
35

143

<210> 188  
<211> 35  
<212> PRT  
<213> Homo sapiens

<400> 188  
Thr Leu Leu Glu Asn Cys Gly Cys Ala Asp Leu Gly Asn Cys Ala Ile  
1 5 10 15  
Lys Pro Glu Thr His Ile Arg Leu Leu Lys Lys Phe Lys Val Val Ala  
20 25 30  
Ser Gly Leu  
35

<210> 189  
<211> 36  
<212> PRT  
<213> Homo sapiens

<400> 189  
Asn Tyr Lys Lys Leu Thr Asp Glu Asn Met Ser Pro Leu Glu Ala Leu  
1 5 10 15  
Glu Pro Val Leu Ser Ser Gln Asn Ile Leu Ser Ile Ser Lys Leu Val  
20 25 30  
Pro Lys Ile Pro  
35

<210> 190  
<211> 36  
<212> PRT  
<213> Homo sapiens

<400> 190  
Glu Lys Asp Gly Gln Met Leu Ser Pro Ser Ser Leu Tyr Thr Ile Trp  
1 5 10 15  
Leu Gln Lys Leu Phe Trp Thr Gly Asp Pro His Leu Ile Lys Gln Val  
20 25 30  
Pro Gly Ser Ser  
35

<210> 191

144

&lt;211&gt; 35

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 191

Pro Glu Trp Leu His Ala Tyr Asp Val Cys Met Lys Tyr Phe Asp Arg  
 1 5 10 15

Leu His Pro Gly Asp Leu Ile Thr Val Val Asp Ala Val Thr Phe Ser  
 20 25 30

Pro Lys Ala  
 35

&lt;210&gt; 192

&lt;211&gt; 39

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 192

Val Thr Lys Leu Ser Val Glu Ala Arg Lys Glu Met Thr Arg Lys Ala  
 1 5 10 15

Ile Lys Thr Val Lys His Phe Ile Glu Lys Pro Lys Glu Lys Lys Leu  
 20 25 30

Arg Arg Arg Ser Ser Arg Ser  
 35

&lt;210&gt; 193

&lt;211&gt; 244

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 193

Met Leu Val Tyr Leu Ile Thr Gly Asp Val Lys Phe Gly Leu Leu Ala  
 1 5 10 15

Arg Val Gly Cys Cys Leu Thr Val Pro Thr Glu Arg Cys Phe Phe Ser  
 20 25 30

Phe Cys Ala Ala Val Lys Lys Pro Ala Pro Ala Pro Pro Lys Pro Gly  
 35 40 45

Asn Pro Pro Pro Gly His Pro Gly Gly Gln Ser Ser Ser Gly Thr Ser  
 50 55 60

Gln His Pro Pro Ser Leu Ser Pro Lys Pro Pro Thr Arg Ser Pro Ser  
 65 70 75 80

145

Pro Pro Thr Gln His Thr Gly Gln Pro Pro Gly Gln Pro Ser Ala Pro  
                     85                    90                    95  
 Ser Gln Leu Ser Ala Pro Arg Arg Tyr Ser Ser Ser Leu Ser Pro Ile  
                     100                    105                    110  
 Gln Ala Pro Asn His Pro Pro Pro Gln Pro Pro Thr Gln Ala Thr Pro  
                     115                    120                    125  
 Leu Met His Thr Lys Pro Asn Ser Gln Gly Pro Pro Asn Pro Met Ala  
                     130                    135                    140  
 Leu Pro Ser Glu His Gly Leu Glu Gln Pro Ser His Thr Pro Pro Gln  
 145                    150                    155                    160  
 Thr Pro Thr Pro Pro Ser Thr Pro Pro Leu Gly Lys Gln Asn Pro Ser  
                     165                    170                    175  
 Leu Pro Ala Pro Gln Thr Leu Ala Gly Gly Asn Pro Glu Thr Ala Gln  
                     180                    185                    190  
 Pro His Ala Gly Thr Leu Pro Arg Pro Arg Pro Val Pro Lys Pro Arg  
                     195                    200                    205  
 Asn Arg Pro Ser Val Pro Pro Pro Pro Gln Pro Pro Gly Val His Ser  
                     210                    215                    220  
 Ala Gly Asp Ser Ser Leu Thr Asn Thr Ala Pro Thr Ala Ser Lys Ile  
 225                    230                    235                    240  
 Val Thr Asp Val

&lt;210&gt; 194

&lt;211&gt; 171

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 194

Phe Ile Phe Ser Val Lys Lys Lys Lys Thr Asp Asp Gly Pro Ser Leu  
   1                    5                    10                    15  
 Gly Ala Gln Asp Gln Arg Ser Thr Pro Thr Asn Gln Lys Gly Ser Ile  
                     20                    25                    30  
 Ile Pro Asn Asn Ile Arg His Lys Phe Gly Ser Asn Val Val Asp Gln  
                     35                    40                    45  
 Leu Val Ser Glu Glu Gln Ala Gln Lys Ala Ile Asp Glu Val Phe Glu

146

50                      55                      60  
 Gly Gln Lys Arg Ala Ser Ser Trp Pro Ser Arg Thr Gln Asn Pro Val  
 65                      70                      75                      80  
 Glu Ile Ser Ser Val Phe Ser Asp Tyr Tyr Asp Leu Gly Tyr Asn Met  
                     85                      90                      95  
 Arg Ser Asn Leu Phe Arg Gly Ala Ala Glu Glu Thr Lys Ser Leu Met  
                     100                      105                      110  
 Lys Ala Ser Tyr Thr Pro Glu Val Ile Glu Lys Ser Val Arg Asp Leu  
                     115                      120                      125  
 Glu His Trp His Gly Arg Lys Thr Asp Asp Leu Gly Arg Trp His Gln  
                     130                      135                      140  
 Lys Asn Ala Met Asn Leu Asn Leu Gln Lys Ala Leu Glu Glu Lys Tyr  
 145                      150                      155                      160  
 Gly Glu Asn Ser Lys Ser Lys Ser Ser Lys Tyr  
                     165                      170

<210> 195  
 <211> 31  
 <212> PRT  
 <213> Homo sapiens

<400> 195  
 Gly Ser Ile Ile Pro Asn Asn Ile Arg His Lys Phe Gly Ser Asn Val  
   1                      5                      10                      15  
 Val Asp Gln Leu Val Ser Glu Glu Gln Ala Gln Lys Ala Ile Asp  
                     20                      25                      30

<210> 196  
 <211> 33  
 <212> PRT  
 <213> Homo sapiens

<400> 196  
 Glu Val Phe Glu Gly Gln Lys Arg Ala Ser Ser Trp Pro Ser Arg Thr  
   1                      5                      10                      15  
 Gln Asn Pro Val Glu Ile Ser Ser Val Phe Ser Asp Tyr Tyr Asp Leu  
                     20                      25                      30

Gly

147

&lt;210&gt; 197

&lt;211&gt; 40

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 197

Tyr Asn Met Arg Ser Asn Leu Phe Arg Gly Ala Ala Glu Glu Thr Lys  
1 5 10 15

Ser Leu Met Lys Ala Ser Tyr Thr Pro Glu Val Ile Glu Lys Ser Val  
20 25 30

Arg Asp Leu Glu His Trp His Gly  
35 40

&lt;210&gt; 198

&lt;211&gt; 38

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 198

Arg Lys Thr Asp Asp Leu Gly Arg Trp His Gln Lys Asn Ala Met Asn  
1 5 10 15

Leu Asn Leu Gln Lys Ala Leu Glu Glu Lys Tyr Gly Glu Asn Ser Lys  
20 25 30

Ser Lys Ser Ser Lys Tyr  
35

&lt;210&gt; 199

&lt;211&gt; 39

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 199

His Glu Ser Ala Arg Gly Arg Trp Glu Gly Gly Gly Arg Arg Ala Cys  
1 5 10 15

Arg Gly Ser Leu Gly Leu Ala Arg Ala Gln Gly Ala Glu Arg Val Thr  
20 25 30

Ser Ser Glu Gln Arg Pro Ala  
35

&lt;210&gt; 200

148

&lt;211&gt; 160

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 200

Ser Gln Val Pro Lys Arg Thr Asp Ser Ser Glu Pro Cys Gly Leu Ser  
 1 5 10 15

Asp Leu Cys Arg Ser Leu Met Thr Lys Pro Gly Cys Ser Gly Tyr Cys  
 20 25 30

Leu Ser His Gln Leu Leu Phe Phe Leu Trp Ala Arg Met Arg Gly Cys  
 35 40 45

Thr Gln Gly Pro Leu Gln Gln Ser Gln Asp Tyr Ile Thr Phe Cys Ala  
 50 55 60

Asn Met Met Asp Leu Asn Arg Arg Ala Glu Ala Ile Gly Tyr Ala Tyr  
 65 70 75 80

Pro Thr Arg Asp Ile Phe Met Glu Asn Ile Met Phe Cys Gly Met Gly  
 85 90 95

Gly Phe Ser Asp Phe Tyr Lys Leu Arg Trp Leu Glu Ala Ile Leu Ser  
 100 105 110

Trp Gln Lys Gln Gln Glu Gly Cys Phe Gly Glu Pro Asp Ala Glu Asp  
 115 120 125

Glu Glu Leu Ser Lys Ala Ile Gln Tyr Gln Gln His Phe Ser Arg Arg  
 130 135 140

Val Lys Arg Arg Glu Lys Gln Phe Pro Glu Tyr Trp Lys Trp Cys Pro  
 145 150 155 160

&lt;210&gt; 201

&lt;211&gt; 39

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 201

Ser Gln Val Pro Lys Arg Thr Asp Ser Ser Glu Pro Cys Gly Leu Ser  
 1 5 10 15

Asp Leu Cys Arg Ser Leu Met Thr Lys Pro Gly Cys Ser Gly Tyr Cys  
 20 25 30

149

Leu Ser His Gln Leu Leu Phe  
35

<210> 202  
<211> 36  
<212> PRT  
<213> Homo sapiens

<400> 202  
Phe Leu Trp Ala Arg Met Arg Gly Cys Thr Gln Gly Pro Leu Gln Gln  
1 5 10 15

Ser Gln Asp Tyr Ile Thr Phe Cys Ala Asn Met Met Asp Leu Asn Arg  
20 25 30

Arg Ala Glu Ala  
35

<210> 203  
<211> 44  
<212> PRT  
<213> Homo sapiens

<400> 203  
Ile Gly Tyr Ala Tyr Pro Thr Arg Asp Ile Phe Met Glu Asn Ile Met  
1 5 10 15

Phe Cys Gly Met Gly Gly Phe Ser Asp Phe Tyr Lys Leu Arg Trp Leu  
20 25 30

Glu Ala Ile Leu Ser Trp Gln Lys Gln Gln Glu Gly  
35 40

<210> 204  
<211> 41  
<212> PRT  
<213> Homo sapiens

<400> 204  
Cys Phe Gly Glu Pro Asp Ala Glu Asp Glu Glu Leu Ser Lys Ala Ile  
1 5 10 15

Gln Tyr Gln Gln His Phe Ser Arg Arg Val Lys Arg Arg Glu Lys Gln  
20 25 30

Phe Pro Glu Tyr Trp Lys Trp Cys Pro  
35 40



150

<210> 205  
 <211> 49  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (25)  
 <223> Xaa equals any of the naturally occurring L-amino acids

<400> 205  
 Lys Ser Val Gly Arg Ser Ser Pro Thr Arg Arg Tyr Arg Ala Ala Val  
   1                  5                  10                  15  
 Gly Glu Thr Pro Ala Gly Ala Gln Xaa Gln Leu Arg Gly Arg Glu Gly  
                   20                  25                  30  
 Arg Trp Arg Arg Leu Gly Gln Pro Phe Pro Arg Gly Ser Thr Ala Leu  
                   35                  40                  45  
 Arg

<210> 206  
 <211> 55  
 <212> PRT  
 <213> Homo sapiens

<400> 206  
 Ile Phe Leu Phe Tyr Leu Pro Pro Ser Pro Pro Ser Arg Leu Leu Val  
   1                  5                  10                  15  
 Pro Gly Tyr Trp Cys Leu Ala Ser Trp Gln Gly Pro Gly Thr Trp Thr  
                   20                  25                  30  
 Ile Ser His Thr Thr Pro Arg Gly Gly Ile Phe Phe Tyr Phe Pro Tyr  
                   35                  40                  45  
 Glu Lys Gln Ile Phe Leu Arg  
                   50                  55

<210> 207  
 <211> 62  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE

151

&lt;222&gt; (1)

&lt;223&gt; Xaa equals any of the naturally occurring L-amino acids

&lt;400&gt; 207

Xaa Arg Gly Met Val Phe Gly Gly Val Val Pro Tyr Val Pro Gln Tyr  
 1 5 10 15

Arg Asp Ile Arg Arg Thr Gln Asn Ala Asp Gly Phe Ser Thr Tyr Val  
 20 25 30

Cys Leu Val Leu Leu Val Ala Asn Ile Leu Arg Ile Leu Phe Trp Phe  
 35 40 45

Gly Arg Arg Phe Glu Ser Pro Leu Leu Trp Gln Ser Ala Ile  
 50 55 60

&lt;210&gt; 208

&lt;211&gt; 383

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (39)

&lt;223&gt; Xaa equals any of the naturally occurring L-amino acids

&lt;400&gt; 208

Arg Thr Gly Trp Leu Gly Pro Pro Gly Ser Pro Pro Pro Pro His  
 1 5 10 15

Val Arg Gly Met Pro Gly Cys Pro Cys Pro Gly Cys Gly Met Ala Gly  
 20 25 30

Pro Arg Leu Leu Phe Leu Xaa Ala Leu Ala Leu Glu Leu Leu Gly Arg  
 35 40 45

Ala Gly Gly Ser Gln Pro Ala Leu Arg Ser Arg Gly Thr Ala Thr Ala  
 50 55 60

Cys Arg Leu Asp Asn Lys Glu Ser Glu Ser Trp Gly Ala Leu Leu Ser  
 65 70 75 80

Gly Glu Arg Leu Asp Thr Trp Ile Cys Ser Leu Leu Gly Ser Leu Met  
 85 90 95

Val Gly Leu Ser Gly Val Phe Pro Leu Leu Val Ile Pro Leu Glu Met  
 100 105 110

Gly Thr Met Leu Arg Ser Glu Ala Gly Ala Trp Arg Leu Lys Gln Leu  
 115 120 125

152

Leu Ser Phe Ala Leu Gly Gly Leu Leu Gly Asn Val Phe Leu His Leu  
130 135 140

Leu Pro Glu Ala Trp Ala Tyr Thr Cys Ser Ala Ser Pro Gly Gly Glu  
145 150 155 160

Gly Gln Ser Leu Gln Gln Gln Gln Gln Leu Gly Leu Trp Val Ile Ala  
165 170 175

Gly Ile Leu Thr Phe Leu Ala Leu Glu Lys Met Phe Leu Asp Ser Lys  
180 185 190

Glu Glu Gly Thr Ser Gln Ala Pro Asn Lys Asp Pro Thr Ala Ala Ala  
195 200 205

Ala Ala Leu Asn Gly Gly His Cys Leu Ala Gln Pro Ala Ala Glu Pro  
210 215 220

Gly Leu Gly Ala Val Val Arg Ser Ile Lys Val Ser Gly Tyr Leu Asn  
225 230 235 240

Leu Leu Ala Asn Thr Ile Asp Asn Phe Thr His Gly Leu Ala Val Ala  
245 250 255

Ala Ser Phe Leu Val Ser Lys Lys Ile Gly Leu Leu Thr Thr Met Ala  
260 265 270

Ile Leu Leu His Glu Ile Pro His Glu Val Gly Asp Phe Ala Ile Leu  
275 280 285

Leu Arg Ala Gly Phe Asp Arg Trp Ser Ala Ala Lys Leu Gln Leu Ser  
290 295 300

Thr Ala Leu Gly Gly Leu Leu Gly Ala Gly Phe Ala Ile Cys Thr Gln  
305 310 315 320

Ser Pro Lys Gly Val Glu Glu Thr Ala Ala Trp Val Leu Pro Phe Thr  
325 330 335

Ser Gly Gly Phe Leu Tyr Ile Ala Leu Val Asn Val Leu Pro Asp Leu  
340 345 350

Leu Glu Glu Glu Asp Pro Trp Arg Ser Leu Gln Gln Leu Leu Leu Leu  
355 360 365

Cys Ala Gly Ile Val Val Met Val Leu Phe Ser Leu Phe Val Asp  
370 375 380

&lt;210&gt; 209

153

&lt;211&gt; 24

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 209

Arg Val Arg Lys Trp Glu Arg Ser Gln Pro Arg Leu Leu Tyr Thr Gly  
1 5 10 15

Lys Leu Ser Gly Pro Gln Ala Arg  
20

&lt;210&gt; 210

&lt;211&gt; 97

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 210

Ser Pro Ala Trp Ala Gln Leu Pro Gln Ser His Pro Leu Pro Thr Ala  
1 5 10 15

Ser Gly Leu Lys Asn Ile Pro Gly Ile Arg Gly Ala Leu Thr Thr Arg  
20 25 30

Pro Ser Glu Ser Pro Pro Ala Trp Asn Leu Ala Ile Ser Asn Leu Leu  
35 40 45

Pro Ser Ala Ser Trp Ile Lys Leu Glu Thr Ala Gly Thr Pro Gly Met  
50 55 60

Ser Leu Pro Ile Leu Pro Cys Leu Cys Ser Phe Leu Asp Leu Thr Tyr  
65 70 75 80

Tyr Phe Phe Cys Phe Cys Phe His Pro Ser Cys Leu Ser Cys Pro Glu  
85 90 95

Gly

&lt;210&gt; 211

&lt;211&gt; 36

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 211

Arg Pro Ser Glu Ser Pro Pro Ala Trp Asn Leu Ala Ile Ser Asn Leu  
1 5 10 15

Leu Pro Ser Ala Ser Trp Ile Lys Leu Glu Thr Ala Gly Thr Pro Gly  
20 25 30

154

Met Ser Leu Pro  
35

<210> 212  
<211> 30  
<212> PRT  
<213> Homo sapiens

<400> 212  
Ile Leu Pro Cys Leu Cys Ser Phe Leu Asp Leu Thr Tyr Tyr Phe Phe  
1 5 10 15  
Cys Phe Cys Phe His Pro Ser Cys Leu Ser Cys Pro Glu Gly  
20 25 30

<210> 213  
<211> 203  
<212> PRT  
<213> Homo sapiens

<400> 213  
Met Gly Arg Asp Ile Pro Gly Val Pro Ala Val Ser Ser Leu Ile Gln  
1 5 10 15  
Glu Ala Leu Gly Arg Arg Leu Leu Met Ala Arg Phe Gln Ala Gly Gly  
20 25 30  
Asp Ser Glu Gly Arg Val Val Asn Ala Pro Leu Ile Pro Gly Ile Phe  
35 40 45  
Phe Arg Pro Glu Ala Val Gly Arg Gly Trp Leu Cys Gly Ser Trp Ala  
50 55 60  
Gln Ala Gly Leu Gln Asn His Pro Leu Trp Gly Asp Asp Gly Gly Gln  
65 70 75 80  
Phe Gln Gly Pro Pro Ala Ile His Trp Ala Val Trp Leu Arg Leu Ser  
85 90 95  
Ala Val Ala Thr Glu Ala Leu Ser Gln Ala Thr Asp Ala Lys Asp Gly  
100 105 110  
Gln Asp Asp Gln Glu Asp Asp Asp Glu Asp Pro His Gly Ala Arg Glu  
115 120 125  
Glu Leu Val Leu Leu Ala Ala Ala Val Thr Thr Ala Phe Glu Ser Phe  
130 135 140

155

Gly Ala Gly Lys Asp Glu Thr Thr Phe Gly Cys Asn Leu Leu Gly Ala  
145 150 155 160

Ser Gln Gln Ala Glu Gln Gln Gly Gly Arg Glu Ala Gly Asp Pro Ser  
165 170 175

Leu Gly His Pro Gly Leu Gly Ala Thr Glu Leu Ser Cys Val Glu Lys  
180 185 190

Ala Gly Leu Arg Pro Leu Pro Leu Pro Asp Ala  
195 200

&lt;210&gt; 214

&lt;211&gt; 13

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 214

Ala Arg Ala Ala Arg Gly Lys Ile Glu Ser Asn Leu Ile  
1 5 10

&lt;210&gt; 215

&lt;211&gt; 10

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 215

Gly Pro Gln Val Asp Trp Gln Arg Pro Leu  
1 5 10

&lt;210&gt; 216

&lt;211&gt; 77

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 216

His Met Leu Trp Asn Arg Arg Lys Leu Arg Cys Cys Phe His Lys Phe  
1 5 10 15

Val Leu Ser Leu Ala Leu Gly Pro Ser Phe Leu Phe Trp Lys Asn Leu  
20 25 30

Ser Glu Lys Arg Asp Leu Ser Ser Val Cys Ser Ala Phe Leu Tyr Lys  
35 40 45

Thr Arg Asn Gly Val Asn Ser Arg Asp Met Glu Val Ile Thr Pro Asp  
50 55 60

156

Ser Leu Cys Trp Leu Leu Arg Phe Ser Gln Gly Glu Val  
 65 70 75

&lt;210&gt; 217

&lt;211&gt; 284

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (187)

&lt;223&gt; Xaa equals any of the naturally occurring L-amino acids

&lt;400&gt; 217

Met Val Thr Arg Ala Gly Ala Gly Thr Ala Val Ala Gly Ala Val Val  
 1 5 10 15

Val Ala Leu Leu Ser Ala Ala Leu Ala Leu Tyr Gly Pro Pro Leu Asp  
 20 25 30

Ala Val Leu Glu Arg Ala Phe Ser Leu Arg Lys Ala His Ser Ile Lys  
 35 40 45

Asp Met Glu Asn Thr Leu Gln Leu Val Arg Asn Ile Ile Pro Pro Leu  
 50 55 60

Ser Ser Thr Lys His Lys Gly Gln Asp Gly Arg Ile Gly Val Val Gly  
 65 70 75 80

Gly Cys Gln Glu Tyr Thr Gly Ala Pro Tyr Phe Ala Arg Ile Ser Ala  
 85 90 95

Leu Lys Val Gly Ala Asp Leu Ser His Val Phe Cys Ala Ser Ala Ala  
 100 105 110

Ala Pro Val Ile Lys Ala Tyr Ser Pro Glu Leu Ile Val His Pro Val  
 115 120 125

Leu Asp Ser Pro Asn Ala Val His Glu Val Glu Lys Trp Leu Pro Arg  
 130 135 140

Leu His Ala Leu Val Val Gly Pro Gly Leu Gly Arg Asp Asp Ala Leu  
 145 150 155 160

Leu Arg Asn Val Gln Gly Ile Leu Glu Val Ser Lys Ala Arg Asp Ile  
 165 170 175

Pro Val Val Ile Asp Ala Asp Gly Leu Trp Xaa Val Ala Gln Gln Pro  
 180 185 190

157

Ala Leu Ile His Gly Tyr Arg Lys Ala Val Leu Thr Pro Asn His Val  
195 200 205

Glu Phe Ser Arg Leu Tyr Asp Ala Val Leu Arg Gly Pro Met Asp Ser  
210 215 220

Asp Asp Ser His Gly Ser Val Leu Arg Leu Ser Gln Ala Leu Gly Asn  
225 230 235 240

Val Thr Val Val Gln Lys Gly Glu Arg Asp Ile Leu Ser Asn Gly Gln  
245 250 255

Gln Val Leu Val Cys Ser Gln Glu Gly Ser Ser Ala Gly Val Glu Gly  
260 265 270

Lys Gly Thr Ser Cys Arg Ala Pro Trp Ala Ser Trp  
275 280

&lt;210&gt; 218

&lt;211&gt; 114

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 218

Met Ala Trp Val Glu Met Ile Val His Pro Val Leu Asp Ser Pro Asn  
1 5 10 15

Ala Val His Glu Val Glu Lys Trp Leu Pro Arg Leu His Ala Leu Val  
20 25 30

Val Gly Thr Gly Leu Gly Arg Asp Asp Ala Leu Leu Arg Asn Val Gln  
35 40 45

Gly Ile Leu Glu Val Ser Lys Ala Arg Asp Ile Pro Val Val Ile Asp  
50 55 60

Ala Asp Gly Leu Trp Leu Val Ala Gln Gln Pro Ala Leu Ile His Gly  
65 70 75 80

Tyr Arg Lys Ala Val Leu Thr Pro Asn His Val Glu Phe Ser Arg Leu  
85 90 95

Tyr Asp Ala Val Leu Arg Gly Pro Met Asp Ser Asp Asp Arg Cys Leu  
100 105 110

Val Pro

&lt;210&gt; 219



158

&lt;211&gt; 202

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 219

Glu Phe Gly Thr Arg Leu Arg Ala Val Ala Ser Val Gly Ala Ala Leu  
 1 5 10 15

Ile Leu Phe Pro Cys Leu Leu Tyr Gly Ala Tyr Ala Phe Leu Pro Phe  
 20 25 30

Asp Val Pro Arg Leu Pro Thr Met Ser Ser Arg Leu Ile Tyr Thr Leu  
 35 40 45

Arg Cys Gly Val Phe Ala Thr Phe Pro Ile Val Leu Gly Ile Leu Val  
 50 55 60

Tyr Gly Leu Ser Leu Leu Cys Phe Ser Ala Leu Arg Pro Phe Gly Glu  
 65 70 75 80

Pro Arg Arg Glu Val Glu Ile His Arg Arg Tyr Val Ala Gln Ser Val  
 85 90 95

Gln Leu Phe Ile Leu Tyr Phe Phe Asn Leu Ala Val Leu Ser Thr Tyr  
 100 105 110

Leu Pro Gln Asp Thr Leu Lys Leu Leu Pro Leu Leu Thr Gly Leu Phe  
 115 120 125

Ala Val Ser Arg Leu Ile Tyr Trp Leu Thr Phe Ala Val Gly Arg Ser  
 130 135 140

Phe Arg Gly Phe Gly Tyr Gly Leu Thr Phe Leu Pro Leu Leu Ser Met  
 145 150 155 160

Leu Met Trp Asn Leu Tyr Tyr Met Phe Val Val Glu Pro Glu Arg Met  
 165 170 175

Leu Thr Ala Thr Glu Ser Arg Leu Asp Tyr Pro Asp His Ala Arg Ser  
 180 185 190

Ala Ser Asp Tyr Arg Pro Arg Pro Trp Gly  
 195 200

&lt;210&gt; 220

&lt;211&gt; 22

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 220

159

Thr Trp Gly His Val His Thr Thr Ala Arg Ala Tyr Cys Val Ser Arg  
 1 5 10 15

Trp Leu Val Cys Leu Arg  
 20

<210> 221  
 <211> 30  
 <212> PRT  
 <213> Homo sapiens

<400> 221  
 Gly Thr Ser Phe Ser Ile Leu Ser Leu Ala Ala Cys Leu Val Val Glu  
 1 5 10 15

Ala Val Val Trp Lys Ser Val Thr Lys Asn Arg Thr Ser Tyr  
 20 25 30

<210> 222  
 <211> 241  
 <212> PRT  
 <213> Homo sapiens

<400> 222  
 His Trp Gly Leu Met Leu Phe Tyr Arg Leu Val Phe Ile Leu His Glu  
 1 5 10 15

Thr Ser Arg Ser Thr Gln Lys Ala Ile Ala Phe Cys Leu Gly Tyr Gly  
 20 25 30

Cys Pro Leu Ala Ile Ser Val Ile Thr Leu Gly Ala Thr Gln Pro Arg  
 35 40 45

Glu Val Tyr Thr Arg Lys Asn Val Cys Trp Leu Asn Trp Glu Asp Thr  
 50 55 60

Lys Ala Leu Leu Ala Phe Ala Ile Pro Ala Leu Ile Ile Val Val Val  
 65 70 75 80

Asn Ile Thr Ile Thr Ile Val Val Ile Thr Lys Ile Leu Arg Pro Ser  
 85 90 95

Ile Gly Asp Lys Pro Cys Lys Gln Glu Lys Ser Ser Leu Phe Gln Ile  
 100 105 110

Ser Lys Ser Ile Gly Val Leu Thr Pro Leu Leu Gly Leu Thr Trp Gly  
 115 120 125

Phe Gly Leu Thr Thr Val Phe Pro Gly Thr Asn Leu Val Phe His Ile

160

130                      135                      140  
 Ile Phe Ala Ile Leu Asn Val Phe Gln Gly Leu Phe Ile Leu Leu Phe  
 145                      150                      155                      160  
 Gly Cys Leu Trp Asp Leu Lys Val Gln Glu Ala Leu Leu Asn Lys Phe  
                     165                      170                      175  
 Ser Leu Ser Arg Trp Ser Ser Gln His Ser Lys Ser Thr Ser Leu Gly  
                     180                      185                      190  
 Ser Ser Thr Pro Val Phe Ser Met Ser Ser Pro Ile Ser Arg Arg Phe  
                     195                      200                      205  
 Asn Asn Leu Phe Gly Lys Thr Gly Thr Tyr Asn Val Ser Thr Pro Glu  
                     210                      215                      220  
 Ala Thr Ser Ser Ser Leu Glu Asn Ser Ser Ser Ala Ser Ser Leu Leu  
 225                      230                      235                      240  
 Asn

<210> 223  
 <211> 36  
 <212> PRT  
 <213> Homo sapiens

<400> 223  
 His Trp Gly Leu Met Leu Phe Tyr Arg Leu Val Phe Ile Leu His Glu  
   1                      5                      10                      15  
 Thr Ser Arg Ser Thr Gln Lys Ala Ile Ala Phe Cys Leu Gly Tyr Gly  
                     20                      25                      30  
 Cys Pro Leu Ala  
                     35

<210> 224  
 <211> 35  
 <212> PRT  
 <213> Homo sapiens

<400> 224  
 Ile Ser Val Ile Thr Leu Gly Ala Thr Gln Pro Arg Glu Val Tyr Thr  
   1                      5                      10                      15  
 Arg Lys Asn Val Cys Trp Leu Asn Trp Glu Asp Thr Lys Ala Leu Leu  
                     20                      25                      30

161

Ala Phe Ala  
35

<210> 225  
<211> 35  
<212> PRT  
<213> Homo sapiens

<400> 225  
Ile Pro Ala Leu Ile Ile Val Val Val Asn Ile Thr Ile Thr Ile Val  
1 5 10 15  
Val Ile Thr Lys Ile Leu Arg Pro Ser Ile Gly Asp Lys Pro Cys Lys  
20 25 30

Gln Glu Lys  
35

<210> 226  
<211> 36  
<212> PRT  
<213> Homo sapiens

<400> 226  
Ser Ser Leu Phe Gln Ile Ser Lys Ser Ile Gly Val Leu Thr Pro Leu  
1 5 10 15  
Leu Gly Leu Thr Trp Gly Phe Gly Leu Thr Thr Val Phe Pro Gly Thr  
20 25 30

Asn Leu Val Phe  
35

<210> 227  
<211> 36  
<212> PRT  
<213> Homo sapiens

<400> 227  
His Ile Ile Phe Ala Ile Leu Asn Val Phe Gln Gly Leu Phe Ile Leu  
1 5 10 15  
Leu Phe Gly Cys Leu Trp Asp Leu Lys Val Gln Glu Ala Leu Leu Asn  
20 25 30

Lys Phe Ser Leu  
35

162

<210> 228  
<211> 35  
<212> PRT  
<213> Homo sapiens

<400> 228  
Ser Arg Trp Ser Ser Gln His Ser Lys Ser Thr Ser Leu Gly Ser Ser  
1 5 10 15  
Thr Pro Val Phe Ser Met Ser Ser Pro Ile Ser Arg Arg Phe Asn Asn  
20 25 30  
Leu Phe Gly  
35

<210> 229  
<211> 28  
<212> PRT  
<213> Homo sapiens

<400> 229  
Lys Thr Gly Thr Tyr Asn Val Ser Thr Pro Glu Ala Thr Ser Ser Ser  
1 5 10 15  
Leu Glu Asn Ser Ser Ser Ala Ser Ser Leu Leu Asn  
20 25

<210> 230  
<211> 14  
<212> PRT  
<213> Homo sapiens

<400> 230  
Thr Arg Pro Leu Trp Ile Pro Arg Ser Leu Val Leu Val Glu  
1 5 10

<210> 231  
<211> 43  
<212> PRT  
<213> Homo sapiens

<400> 231  
Glu Lys Val Gly Leu Leu Pro Thr Thr Ile Ala Ile Ile Gln Ile Ile  
1 5 10 15  
Ser Lys Asp Ser Val Ser Ala Ile Ser Asp Ser Cys Leu Arg Pro Ser

30

Pro Gly Gln Pro Asp Ser Tyr Phe Leu Ser Gly Glu Asn Cys Val Val  
195 200 205

164

Thr Arg Ala  
210

<210> 233  
<211> 42  
<212> PRT  
<213> Homo sapiens

<400> 233  
Arg Gly Glu Ser Glu Glu Thr Gly Ser Ser Glu Gly Ala Pro Ser Leu  
1 5 10 15  
Leu Pro Ala Thr Arg Ala Pro Glu Gly Thr Arg Glu Leu Glu Ala Pro  
20 25 30  
Ser Glu Asp Asn Ser Gly Arg Thr Ala Pro  
35 40

<210> 234  
<211> 40  
<212> PRT  
<213> Homo sapiens

<400> 234  
Ala Gly Thr Ser Val Gln Ala Gln Pro Val Leu Pro Thr Asp Ser Ala  
1 5 10 15  
Ser Arg Gly Gly Val Ala Val Val Pro Ala Ser Gly Asp Cys Val Pro  
20 25 30  
Ser Pro Cys His Asn Gly Gly Thr  
35 40

<210> 235  
<211> 43  
<212> PRT  
<213> Homo sapiens

<400> 235  
Cys Leu Glu Glu Glu Gly Val Arg Cys Leu Cys Leu Pro Gly Tyr  
1 5 10 15  
Gly Gly Asp Leu Cys Asp Val Gly Leu Arg Phe Cys Asn Pro Gly Trp  
20 25 30  
Asp Ala Phe Gln Gly Ala Cys Tyr Lys His Phe  
35 40

165

<210> 236  
<211> 43  
<212> PRT  
<213> Homo sapiens

<400> 236  
Ser Thr Arg Arg Ser Trp Glu Glu Ala Glu Thr Gln Cys Arg Met Tyr  
1 5 10 15  
Gly Ala His Leu Ala Ser Ile Ser Thr Pro Glu Glu Gln Asp Phe Ile  
20 25 30  
Asn Asn Arg Tyr Arg Glu Tyr Gln Trp Ile Gly  
35 40

<210> 237  
<211> 43  
<212> PRT  
<213> Homo sapiens

<400> 237  
Leu Asn Asp Arg Thr Ile Glu Gly Asp Phe Leu Trp Ser Asp Gly Val  
1 5 10 15  
Pro Leu Leu Tyr Glu Asn Trp Asn Pro Gly Gln Pro Asp Ser Tyr Phe  
20 25 30  
Leu Ser Gly Glu Asn Cys Val Val Thr Arg Ala  
35 40

<210> 238  
<211> 81  
<212> PRT  
<213> Homo sapiens

<400> 238  
Gly Thr Arg Ser Ser His Val Pro Ile Ser Asp Ser Lys Ser Ile Gln  
1 5 10 15  
Lys Ser Glu Leu Leu Gly Leu Leu Lys Thr Tyr Asn Cys Tyr His Glu  
20 25 30  
Gly Lys Ser Phe Gln Leu Arg His Arg Glu Glu Glu Gly Thr Leu Ile  
35 40 45  
Ile Glu Gly Leu Leu Asn Ile Ala Trp Gly Leu Arg Arg Pro Ile Arg  
50 55 60



166

Leu Gln Met Gln Asp Asp Arg Glu Gln Val His Leu Pro Ser Thr Ser  
 65 70 75 80

Trp

<210> 239  
 <211> 25  
 <212> PRT  
 <213> Homo sapiens

<400> 239  
 Val Pro Ile Ser Asp Ser Lys Ser Ile Gln Lys Ser Glu Leu Leu Gly  
 1 5 10 15

Leu Leu Lys Thr Tyr Asn Cys Tyr His  
 20 25

<210> 240  
 <211> 28  
 <212> PRT  
 <213> Homo sapiens

<400> 240  
 Phe Gln Leu Arg His Arg Glu Glu Glu Gly Thr Leu Ile Ile Glu Gly  
 1 5 10 15

Leu Leu Asn Ile Ala Trp Gly Leu Arg Arg Pro Ile  
 20 25

<210> 241  
 <211> 344  
 <212> PRT  
 <213> Homo sapiens

<400> 241  
 Gly Thr Arg Ser Ser His Val Pro Ile Ser Asp Ser Lys Ser Ile Gln  
 1 5 10 15

Lys Ser Glu Leu Leu Gly Leu Leu Lys Thr Tyr Asn Cys Tyr His Glu  
 20 25 30

Gly Lys Ser Phe Gln Leu Arg His Arg Glu Glu Glu Gly Thr Leu Ile  
 35 40 45

Ile Glu Gly Leu Leu Asn Ile Ala Trp Gly Leu Arg Arg Pro Ile Arg  
 50 55 60

167

Leu Gln Met Gln Asp Asp Arg Glu Gln Val His Leu Pro Ser Thr Ser  
 65 70 75 80

Trp Met Pro Arg Arg Pro Ser Cys Pro Leu Gly Cys Trp Ser Leu Leu  
 85 90 95

Leu Gly Leu Ser Ser Leu Ser Leu Pro Ala Ala Ile Ser Ala Leu Gln  
 100 105 110

Leu Ser Val Phe Arg Lys Glu Pro Ser Pro Gln Asn Gly Asn Ile Thr  
 115 120 125

Ala Gln Gly Pro Ser Ile Gln Pro Val His Lys Ala Glu Ser Ser Thr  
 130 135 140

Asp Ser Ser Gly Pro Leu Glu Glu Ala Glu Glu Ala Pro Gln Leu Met  
 145 150 155 160

Arg Thr Lys Ser Asp Ala Ser Cys Met Ser Gln Arg Arg Pro Lys Cys  
 165 170 175

Arg Ala Pro Gly Glu Ala Gln Arg Ile Arg Arg His Arg Phe Ser Ile  
 180 185 190

Asn Gly His Phe Tyr Asn His Lys Thr Ser Val Phe Thr Pro Ala Tyr  
 195 200 205

Gly Ser Val Thr Asn Val Arg Val Asn Ser Thr Met Thr Thr Leu Gln  
 210 215 220

Val Leu Thr Leu Leu Leu Asn Lys Phe Arg Val Glu Asp Gly Pro Ser  
 225 230 235 240

Glu Phe Ala Leu Tyr Ile Val His Glu Ser Gly Glu Arg Thr Lys Leu  
 245 250 255

Lys Asp Cys Glu Tyr Pro Leu Ile Ser Arg Ile Leu His Gly Pro Cys  
 260 265 270

Glu Lys Ile Ala Arg Ile Phe Leu Met Glu Ala Asp Leu Gly Val Glu  
 275 280 285

Val Pro His Glu Val Ala Gln Tyr Ile Lys Phe Glu Met Pro Val Leu  
 290 295 300

Asp Ser Phe Val Glu Lys Leu Lys Glu Glu Glu Glu Arg Glu Ile Ile  
 305 310 315 320

Lys Leu Thr Met Lys Phe Gln Ala Leu Arg Leu Thr Met Leu Gln Arg  
 325 330 335

168

Leu Glu Gln Leu Val Glu Ala Lys  
340

&lt;210&gt; 242

&lt;211&gt; 86

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 242

His Ala Ser Gly Phe Leu Arg Phe Cys Lys Gln Ala Thr Leu Gln Phe  
1 5 10 15

Cys Val Val Lys Pro Leu Met Ala Val Ser Thr Val Val Leu Gln Ala  
20 25 30

Phe Gly Lys Tyr Arg Asp Gly Asp Phe Asp Val Thr Ser Gly Tyr Leu  
35 40 45

Tyr Val Thr Ile Ile Tyr Asn Ile Ser Val Ser Leu Ala Leu Tyr Ala  
50 55 60

Leu Phe Leu Phe Tyr Phe Ala Thr Arg Glu Leu Leu Ser Pro Tyr Ser  
65 70 75 80

Pro Val Leu Lys Phe Phe  
85

&lt;210&gt; 243

&lt;211&gt; 243

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 243

Thr Arg Thr Thr Ser Cys Arg Thr Pro Ser Thr Thr Ser His Leu Pro  
1 5 10 15

Thr Ser Ser Thr Arg Ser Ser Pro Pro Trp Ser Leu Gly Pro Pro Gly  
20 25 30

Val Val Ala Pro Thr Ala Ser Pro Ala Pro Thr Ala Ser Val Ala Pro  
35 40 45

Ala Thr Thr Arg Arg Leu Ser Cys Ser Ala Leu Met Met Asn Ser Arg  
50 55 60

Cys Gly Leu Gln Trp Arg Lys Cys Trp Arg His Ser His Gly Gln Ala  
65 70 75 80

Val Pro His Leu Gln Pro His His Gln Ala Arg Arg Gln Leu Ala Gln

169

	85		90		95
Cys Ser Arg Arg Leu Tyr Leu Leu Asp Gln Lys His Ser His Val Ala					
	100		105		110
Ser Arg Gly Thr Gly Asp Ser Gln Ala Arg Pro Trp Ala Phe Arg Asn					
	115		120		125
Ile Tyr Thr Trp Pro Ser Leu His Cys Pro Gly Glu Gly Arg Gly His					
	130		135		140
Trp Glu Gln Gly Leu Cys Pro Cys Cys Pro Ser Cys Ala Gly Gly Met					
	145		150		155
Leu Gly Pro Ala Ala Pro Arg Pro Gln Cys Leu Cys Val Asp Gln Arg					
	165		170		175
Leu Gln Pro Ser Ser Pro Ser Ser Pro Arg Asp Ser Gln Ala Glu Val					
	180		185		190
Gly Lys Pro Trp Leu Pro His Thr Pro Cys Asn Thr Leu Ser Asp Leu					
	195		200		205
Gly Ser Ser Arg Leu His Pro Phe Pro Val His Leu Cys Pro Val Leu					
	210		215		220
Asp Ser Pro His Pro Gly Gln Glu Trp Gly Cys Gly Arg Ser Val Val					
	225		230		235
					240
Leu Pro Ser					

&lt;210&gt; 244

&lt;211&gt; 162

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 244

Ile Leu Gly Ala Gly Cys Ser Gly Gly Ser Ala Gly Ala Ile Ala Thr					
1	5		10		15
Val Arg Leu Cys Pro Thr Ser Ser Leu Thr Thr Arg Pro Gly Gly Ser					
	20		25		30
Trp His Ser Ala His Ala Ala Phe Ile Tyr Trp Thr Arg Asn Thr His					
	35		40		45
Met Ser Leu Pro Glu Glu Arg Gly Thr Ala Arg Leu Ala His Gly Pro					
	50		55		60

170

Ser Gly Ile Phe Ile His Gly Pro Ala Cys Thr Ala Arg Ala Arg Ala  
65 70 75 80

Glu Asp Thr Gly Ser Lys Ala Tyr Ala Pro Ala Ala Arg Pro Val Leu  
85 90 95

Gly Ala Cys Trp Asp Gln Pro His Pro Gly Pro Asn Ala Cys Val Trp  
100 105 110

Thr Ser Gly Cys Ser Leu Leu Ala Pro Pro Pro Arg Glu Thr Leu Arg  
115 120 125

Leu Arg Ser Ala Ser Arg Gly Ser Pro Thr His Arg Ala Ile Pro Cys  
130 135 140

Leu Thr Trp Ala Leu Pro Ala Cys Ile Pro Ser Leu Ser Thr Phe Val  
145 150 155 160

Gln Cys

<210> 245

<211> 35

<212> PRT

<213> Homo sapiens

<400> 245

Thr Arg Thr Thr Ser Cys Arg Thr Pro Ser Thr Thr Ser His Leu Pro  
1 5 10 15

Thr Ser Ser Thr Arg Ser Ser Pro Pro Trp Ser Leu Gly Pro Pro Gly  
20 25 30

Val Val Ala  
35

<210> 246

<211> 36

<212> PRT

<213> Homo sapiens

<400> 246

Pro Thr Ala Ser Pro Ala Pro Thr Ala Ser Val Ala Pro Ala Thr Thr  
1 5 10 15

Arg Arg Leu Ser Cys Ser Ala Leu Met Met Asn Ser Arg Cys Gly Leu  
20 25 30

Gln Trp Arg Lys

171

35

&lt;210&gt; 247

&lt;211&gt; 36

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 247

Cys Trp Arg His Ser His Gly Gln Ala Val Pro His Leu Gln Pro His

1

5

10

15

His Gln Ala Arg Arg Gln Leu Ala Gln Cys Ser Arg Arg Leu Tyr Leu

20

25

30

Leu Asp Gln Lys

35

&lt;210&gt; 248

&lt;211&gt; 35

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 248

His Ser His Val Ala Ser Arg Gly Thr Gly Asp Ser Gln Ala Arg Pro

1

5

10

15

Trp Ala Phe Arg Asn Ile Tyr Thr Trp Pro Ser Leu His Cys Pro Gly

20

25

30

Glu Gly Arg

35

&lt;210&gt; 249

&lt;211&gt; 36

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 249

Gly His Trp Glu Gln Gly Leu Cys Pro Cys Cys Pro Ser Cys Ala Gly

1

5

10

15

Gly Met Leu Gly Pro Ala Ala Pro Arg Pro Gln Cys Leu Cys Val Asp

20

25

30

Gln Arg Leu Gln

35

172

&lt;210&gt; 250

&lt;211&gt; 35

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 250

Pro Ser Ser Pro Ser Ser Pro Arg Asp Ser Gln Ala Glu Val Gly Lys  
1 5 10 15

Pro Trp Leu Pro His Thr Pro Cys Asn Thr Leu Ser Asp Leu Gly Ser  
20 25 30

Ser Arg Leu  
35

&lt;210&gt; 251

&lt;211&gt; 30

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 251

His Pro Phe Pro Val His Leu Cys Pro Val Leu Asp Ser Pro His Pro  
1 5 10 15

Gly Gln Glu Trp Gly Cys Gly Arg Ser Val Val Leu Pro Ser  
20 25 30

&lt;210&gt; 252

&lt;211&gt; 38

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 252

Ile Leu Gly Ala Gly Cys Ser Gly Gly Ser Ala Gly Ala Ile Ala Thr  
1 5 10 15

Val Arg Leu Cys Pro Thr Ser Ser Leu Thr Thr Arg Pro Gly Gly Ser  
20 25 30

Trp His Ser Ala His Ala  
35

&lt;210&gt; 253

&lt;211&gt; 36

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 253

173

Ala Phe Ile Tyr Trp Thr Arg Asn Thr His Met Ser Leu Pro Glu Glu  
1 5 10 15

Arg Gly Thr Ala Arg Leu Ala His Gly Pro Ser Gly Ile Phe Ile His  
20 25 30

Gly Pro Ala Cys  
35

&lt;210&gt; 254

&lt;211&gt; 34

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 254

Thr Ala Arg Ala Arg Ala Glu Asp Thr Gly Ser Lys Ala Tyr Ala Pro  
1 5 10 15

Ala Ala Arg Pro Val Leu Gly Ala Cys Trp Asp Gln Pro His Pro Gly  
20 25 30

Pro Asn

&lt;210&gt; 255

&lt;211&gt; 54

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 255

Ala Cys Val Trp Thr Ser Gly Cys Ser Leu Leu Ala Pro Pro Pro Arg  
1 5 10 15

Glu Thr Leu Arg Leu Arg Ser Ala Ser Arg Gly Ser Pro Thr His Arg  
20 25 30

Ala Ile Pro Cys Leu Thr Trp Ala Leu Pro Ala Cys Ile Pro Ser Leu  
35 40 45

Ser Thr Phe Val Gln Cys  
50

&lt;210&gt; 256

&lt;211&gt; 46

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;



174

&lt;221&gt; SITE

&lt;222&gt; (42)

&lt;223&gt; Xaa equals any of the naturally occurring L-amino acids

&lt;400&gt; 256

Pro His Arg Pro Pro Thr Pro Gln Ser Asn Phe Ser Ser His Pro Ser  
 1 5 10 15

Ser Gln Ala Leu Thr Ile Leu Lys Arg Leu Val Gly Thr Leu Leu Ser  
 20 25 30

Ala Thr Gly Lys Leu Val Arg Ala Arg Xaa Arg Ala Trp Gly  
 35 40 45

&lt;210&gt; 257

&lt;211&gt; 102

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 257

Gly Val Met Arg Leu Arg Thr Arg Gln Lys Ser Arg Arg Gln Arg Lys  
 1 5 10 15

Glu Lys Met Ser Arg Arg Lys Ser Lys Arg Lys Met Lys Arg Lys Arg  
 20 25 30

Arg Arg Arg Gln Arg Ala Arg Gly Gln Ser Gln Pro Met Arg Leu Ser  
 35 40 45

Phe His Pro Phe Pro Thr Leu Val Phe Phe Gln Val Leu Thr Gln Ser  
 50 55 60

Trp Val Leu Ser Ser Arg Arg Gln Leu Leu Val Val Arg Ala Gly Pro  
 65 70 75 80

His Pro Pro Trp Pro Leu Phe Asp Leu Pro His Ser Val Thr Pro Gln  
 85 90 95

Ala Ser His Thr Ser Val  
 100

&lt;210&gt; 258

&lt;211&gt; 43

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 258

Met Lys Arg Lys Arg Arg Arg Gln Arg Ala Arg Gly Gln Ser Gln  
 1 5 10 15

175

Pro Met Arg Leu Ser Phe His Pro Phe Pro Thr Leu Val Phe Phe Gln  
                   20                                  25                                  30

Val Leu Thr Gln Ser Trp Val Leu Ser Ser Arg  
                   35                                  40

&lt;210&gt; 259

&lt;211&gt; 32

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 259

Arg Gln Leu Leu Val Val Arg Ala Gly Pro His Pro Pro Trp Pro Leu  
   1                                  5                                  10                                  15

Phe Asp Leu Pro His Ser Val Thr Pro Gln Ala Ser His Thr Ser Val  
                   20                                  25                                  30

&lt;210&gt; 260

&lt;211&gt; 52

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 260

His His Cys Pro Ala Leu Gln Pro Gly Thr His Thr His Thr His Thr  
   1                                  5                                  10                                  15

His Thr His Thr His Thr Arg Arg Gly Met Cys Leu Val Gln Ile Tyr  
                   20                                  25                                  30

Ile Lys Leu Thr His Arg Gln Ile Pro Cys Leu Cys Leu Leu Gly Pro  
                   35                                  40                                  45

Asp Ser Ala Val  
                   50

&lt;210&gt; 261

&lt;211&gt; 8

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 261

His Glu Ile Leu Gln Pro Ala Val  
   1                                  5

176

&lt;210&gt; 262

&lt;211&gt; 54

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 262

Asn Ser Arg Val Asp Pro Arg Val Arg Asp Gly Leu Met Tyr Gln Lys  
1 5 10 15

Phe Arg Asn Gln Phe Leu Ser Phe Ser Met Tyr Gln Ser Phe Val Gln  
20 25 30

Phe Leu Gln Tyr Tyr Tyr Gln Ser Gly Cys Leu Tyr Arg Leu Arg Ala  
35 40 45

Leu Gly Glu Arg His Thr  
50

&lt;210&gt; 263

&lt;211&gt; 9

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 263

Ile Leu Met Pro Phe Cys Gly Leu His  
1 5

&lt;210&gt; 264

&lt;211&gt; 17

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 264

Leu Pro Leu Val Leu Pro Pro Thr Pro Pro Pro Pro Trp Leu Pro Ser  
1 5 10 15

Leu

&lt;210&gt; 265

&lt;211&gt; 220

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 265

Thr Thr Met Tyr Ala Leu Trp Arg Thr Gly Pro Thr Thr Ser Pro Ala

177

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      1              5              10              15
Leu Leu Thr Leu Leu Ser Lys Gly Val Pro Arg Pro Ala Ala Pro Trp
      20              25              30
Thr Met Ser Pro Ser Ser Val Ala Leu Ile Cys Leu Leu Arg Tyr Gly
      35              40              45
Gln Leu Leu Glu Gln Ser Arg His Ser Trp Val Asn Thr Thr Ala Leu
      50              55              60
Ile Thr Gly Cys Thr Asn Ala Ala Gly Leu Leu Val Val Gly Asn Phe
      65              70              75              80
Gln Val Asp His Ala Arg Ser Leu His Tyr Val Gly Ala Gly Val Ala
      85              90              95
Phe Pro Ala Gly Leu Leu Phe Val Cys Leu His Cys Ala Leu Ser Tyr
      100             105             110
Gln Gly Ala Thr Ala Pro Leu Asp Leu Ala Val Ala Tyr Leu Arg Ser
      115             120             125
Val Leu Ala Val Ile Ala Phe Ile Thr Leu Val Leu Ser Gly Val Phe
      130             135             140
Phe Val His Glu Ser Ser Gln Leu Gln His Gly Ala Ala Leu Cys Glu
      145             150             155             160
Trp Val Cys Val Ile Asp Ile Leu Ile Phe Tyr Gly Thr Phe Ser Tyr
      165             170             175
Glu Phe Gly Ala Val Ser Ser Asp Thr Leu Val Ala Ala Leu Gln Pro
      180             185             190
Thr Pro Gly Arg Ala Cys Lys Ser Ser Gly Ser Ser Ser Thr Ser Thr
      195             200             205
His Leu Asn Cys Ala Pro Glu Ser Ile Ala Met Ile
      210             215             220

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&lt;210&gt; 266

&lt;211&gt; 37

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 266

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Thr Thr Met Tyr Ala Leu Trp Arg Thr Gly Pro Thr Thr Ser Pro Ala
  1              5              10              15

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178

Leu Leu Thr Leu Leu Ser Lys Gly Val Pro Arg Pro Ala Ala Pro Trp  
20 25 30

Thr Met Ser Pro Ser  
35

<210> 267  
<211> 34  
<212> PRT  
<213> Homo sapiens

<400> 267  
Ser Val Ala Leu Ile Cys Leu Leu Arg Tyr Gly Gln Leu Leu Glu Gln  
1 5 10 15

Ser Arg His Ser Trp Val Asn Thr Thr Ala Leu Ile Thr Gly Cys Thr  
20 25 30

Asn Ala

<210> 268  
<211> 37  
<212> PRT  
<213> Homo sapiens

<400> 268  
Ala Gly Leu Leu Val Val Gly Asn Phe Gln Val Asp His Ala Arg Ser  
1 5 10 15

Leu His Tyr Val Gly Ala Gly Val Ala Phe Pro Ala Gly Leu Leu Phe  
20 25 30

Val Cys Leu His Cys  
35

<210> 269  
<211> 34  
<212> PRT  
<213> Homo sapiens

<400> 269  
Ala Leu Ser Tyr Gln Gly Ala Thr Ala Pro Leu Asp Leu Ala Val Ala  
1 5 10 15

Tyr Leu Arg Ser Val Leu Ala Val Ile Ala Phe Ile Thr Leu Val Leu  
20 25 30

179

Ser Gly

&lt;210&gt; 270

&lt;211&gt; 41

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 270

Val Phe Phe Val His Glu Ser Ser Gln Leu Gln His Gly Ala Ala Leu  
1 5 10 15

Cys Glu Trp Val Cys Val Ile Asp Ile Leu Ile Phe Tyr Gly Thr Phe  
20 25 30

Ser Tyr Glu Phe Gly Ala Val Ser Ser  
35 40

&lt;210&gt; 271

&lt;211&gt; 37

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 271

Asp Thr Leu Val Ala Ala Leu Gln Pro Thr Pro Gly Arg Ala Cys Lys  
1 5 10 15

Ser Ser Gly Ser Ser Ser Thr Ser Thr His Leu Asn Cys Ala Pro Glu  
20 25 30

Ser Ile Ala Met Ile  
35

&lt;210&gt; 272

&lt;211&gt; 177

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 272

Ser Ala Ser Cys Ala Thr Gly Ser Ser Trp Ser Arg Val Gly Thr Leu  
1 5 10 15

Gly Leu Thr Pro Arg His Ser Ser Gln Ala Ala Pro Thr Leu Arg Ala  
20 25 30

Ser Trp Trp Leu Ala Thr Phe Arg Trp Ile Met Pro Gly Leu Cys Thr  
35 40 45

180

Thr Leu Glu Leu Ala Trp Pro Ser Leu Arg Gly Cys Ser Leu Phe Ala  
 50 55 60  
 Cys Thr Val Leu Ser Pro Thr Lys Gly Pro Pro Pro Arg Trp Thr Trp  
 65 70 75 80  
 Leu Trp Pro Ile Cys Glu Val Cys Trp Leu Ser Ser Pro Leu Ser Pro  
 85 90 95  
 Trp Ser Ser Val Glu Ser Ser Leu Ser Met Arg Val Leu Ser Cys Asn  
 100 105 110  
 Met Gly Gln Pro Cys Val Ser Gly Cys Val Ser Ser Ile Ser Ser Phe  
 115 120 125  
 Ser Met Ala Pro Ser Ala Thr Ser Leu Gly Gln Ser Pro Gln Thr His  
 130 135 140  
 Trp Trp Leu His Cys Ser Leu Pro Leu Ala Gly Pro Ala Ser Pro Pro  
 145 150 155 160  
 Gly Ala Ala Ala Pro Pro Pro Thr Ser Thr Val Pro Pro Arg Ala Ser  
 165 170 175  
 Leu

<210> 273  
 <211> 38  
 <212> PRT  
 <213> Homo sapiens

<400> 273  
 Ser Ala Ser Cys Ala Thr Gly Ser Ser Trp Ser Arg Val Gly Thr Leu  
 1 5 10 15  
 Gly Leu Thr Pro Arg His Ser Ser Gln Ala Ala Pro Thr Leu Arg Ala  
 20 25 30  
 Ser Trp Trp Leu Ala Thr  
 35

<210> 274  
 <211> 33  
 <212> PRT  
 <213> Homo sapiens

<400> 274  
 Phe Arg Trp Ile Met Pro Gly Leu Cys Thr Thr Leu Glu Leu Ala Trp

181

1                      5                      10                      15  
 Pro Ser Leu Arg Gly Cys Ser Leu Phe Ala Cys Thr Val Leu Ser Pro  
                          20                      25                      30

Thr

<210> 275  
 <211> 36  
 <212> PRT  
 <213> Homo sapiens

<400> 275  
 Lys Gly Pro Pro Pro Arg Trp Thr Trp Leu Trp Pro Ile Cys Glu Val  
   1                      5                      10                      15  
 Cys Trp Leu Ser Ser Pro Leu Ser Pro Trp Ser Ser Val Glu Ser Ser  
                          20                      25                      30

Leu Ser Met Arg  
                          35

<210> 276  
 <211> 35  
 <212> PRT  
 <213> Homo sapiens

<400> 276  
 Val Leu Ser Cys Asn Met Gly Gln Pro Cys Val Ser Gly Cys Val Ser  
   1                      5                      10                      15  
 Ser Ile Ser Ser Phe Ser Met Ala Pro Ser Ala Thr Ser Leu Gly Gln  
                          20                      25                      30

Ser Pro Gln  
                          35

<210> 277  
 <211> 35  
 <212> PRT  
 <213> Homo sapiens

<400> 277  
 Thr His Trp Trp Leu His Cys Ser Leu Pro Leu Ala Gly Pro Ala Ser  
   1                      5                      10                      15  
 Pro Pro Gly Ala Ala Ala Pro Pro Pro Thr Ser Thr Val Pro Pro Arg



182

20

25

30

Ala Ser Leu

35

&lt;210&gt; 278

&lt;211&gt; 15

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 278

Ser Cys His Ser Gly Gln Gln Ser Glu Thr Val Ser Glu Lys Lys

1

5

10

15

&lt;210&gt; 279

&lt;211&gt; 15

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 279

Ser Pro Pro Ile Ser Phe Thr Leu Thr Ser Gly Leu Pro Asn Pro

1

5

10

15

&lt;210&gt; 280

&lt;211&gt; 80

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (15)

&lt;223&gt; Xaa equals any of the naturally occurring L-amino acids

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (16)

&lt;223&gt; Xaa equals any of the naturally occurring L-amino acids

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (24)

&lt;223&gt; Xaa equals any of the naturally occurring L-amino acids

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (70)

&lt;223&gt; Xaa equals any of the naturally occurring L-amino acids

183

&lt;400&gt; 280

Gln Phe His Thr Gly Asn Ser Tyr Asp His Asp Tyr Ala Lys Xaa Xaa  
1 5 10 15  
Tyr Gly Asn Leu Tyr Tyr Arg Xaa Ser Trp Tyr Ala Cys Arg Tyr Arg  
20 25 30  
Ser Gly Ile Pro Gly Ser Thr His Ala Ser Glu Lys Ile Phe Leu Ser  
35 40 45  
Lys Leu Ile Val Cys Phe Leu Ser Thr Trp Leu Pro Phe Val Leu Leu  
50 55 60  
Gln Val Ile Ile Val Xaa Leu Lys Val Gln Ile Pro Ala Tyr Ile Glu  
65 70 75 80

&lt;210&gt; 281

&lt;211&gt; 21

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 281

Ile Pro Ile Arg Phe Val Asn Ile Phe Phe His Ser Ala Gly Cys Leu  
1 5 10 15  
Phe Ile Phe Leu Ile  
20

&lt;210&gt; 282

&lt;211&gt; 659

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 282

Xaa Xaa Xaa Xaa Tyr Arg Ile Pro Leu Ala Ala Asp Ala Gly Leu Leu  
1 5 10 15  
Gln Phe Leu Gln Glu Phe Ser Gln Gln Thr Ile Ser Arg Thr His Glu  
20 25 30  
Ile Lys Lys Gln Val Asp Gly Leu Ile Arg Glu Thr Lys Ala Thr Asp  
35 40 45  
Cys Arg Leu His Asn Val Phe Asn Asp Phe Leu Met Leu Ser Asn Thr  
50 55 60

184

Gln Phe Ile Glu Asn Arg Val Tyr Asp Glu Glu Val Glu Glu Pro Val  
 65 70 75 80

Leu Lys Ala Glu Ala Glu Lys Thr Glu Gln Glu Lys Thr Arg Glu Gln  
 85 90 95

Lys Glu Val Asp Leu Ile Pro Lys Val Gln Glu Ala Val Asn Tyr Gly  
 100 105 110

Leu Gln Val Leu Asp Ser Ala Phe Glu Gln Leu Asp Ile Lys Ala Gly  
 115 120 125

Asn Ser Asp Ser Glu Glu Asp Asp Ala Asn Gly Arg Val Glu Leu Ile  
 130 135 140

Leu Glu Pro Lys Asp Leu Tyr Ile Asp Arg Pro Leu Pro Tyr Leu Ile  
 145 150 155 160

Gly Ser Lys Leu Phe Met Glu Gln Glu Asp Val Gly Leu Gly Glu Leu  
 165 170 175

Ser Ser Glu Glu Gly Ser Val Gly Ser Asp Arg Gly Ser Ile Val Asp  
 180 185 190

Thr Glu Glu Glu Lys Glu Glu Glu Glu Ser Asp Glu Asp Phe Ala His  
 195 200 205

His Ser Asp Asn Glu Gln Asn Gln His Thr Thr Gln Met Ser Asp Glu  
 210 215 220

Glu Glu Asp Asp Asp Gly Cys Asp Leu Phe Ala Asp Ser Glu Lys Glu  
 225 230 235 240

Glu Glu Asp Ile Glu Asp Ile Glu Glu Asn Thr Arg Pro Lys Arg Ser  
 245 250 255

Arg Pro Thr Ser Phe Ala Asp Glu Leu Ala Ala Arg Ile Lys Gly Asp  
 260 265 270

Ala Met Gly Arg Val Asp Glu Glu Pro Thr Thr Leu Pro Ser Gly Glu  
 275 280 285

Ala Lys Pro Arg Lys Thr Leu Lys Glu Lys Lys Glu Arg Arg Thr Pro  
 290 295 300

Ser Asp Asp Glu Glu Asp Asn Leu Phe Ala Pro Pro Lys Leu Thr Asp  
 305 310 315 320

Glu Asp Phe Ser Pro Phe Gly Ser Gly Gly Gly Leu Phe Ser Gly Gly  
 325 330 335

185

Lys Gly Leu Phe Asp Asp Glu Asp Glu Glu Ser Asp Leu Phe Met Glu  
 340 345 350

Ala Pro Gln Asp Arg Gln Ala Gly Ala Ser Val Lys Glu Glu Ser Ser  
 355 360 365

Ser Ser Lys Pro Gly Lys Lys Ile Pro Ala Gly Ala Val Ser Val Phe  
 370 375 380

Leu Gly Asp Thr Asp Val Phe Gly Ala Ala Ser Val Pro Ser Leu Lys  
 385 390 395 400

Glu Pro Gln Lys Pro Glu Gln Pro Thr Pro Arg Lys Ser Pro Tyr Gly  
 405 410 415

Pro Pro Pro Thr Gly Leu Phe Asp Asp Asp Asp Gly Asp Asp Asp Asp  
 420 425 430

Asp Phe Phe Ser Ala Pro His Ser Lys Pro Ser Lys Thr Arg Lys Val  
 435 440 445

Gln Ser Thr Ala Asp Ile Phe Gly Asp Glu Glu Gly Asp Leu Phe Lys  
 450 455 460

Glu Lys Ala Val Ala Ser Pro Glu Ala Thr Val Ser Gln Thr Asp Glu  
 465 470 475 480

Asn Lys Ala Arg Ala Glu Lys Lys Asp Leu Phe Ser Ser Gln Ser Ala  
 485 490 495

Ser Asn Leu Lys Gly Ala Ser Leu Leu Pro Gly Lys Leu Pro Thr Ser  
 500 505 510

Val Ser Leu Phe Asp Asp Glu Asp Glu Glu Asp Asn Leu Phe Gly Gly  
 515 520 525

Thr Ala Ala Lys Lys Gln Thr Leu Ser Leu Gln Ala Gln Arg Glu Glu  
 530 535 540

Lys Ala Lys Ala Ser Glu Leu Ser Lys Lys Lys Ala Ser Ala Leu Leu  
 545 550 555 560

Phe Ser Ser Asp Glu Glu Asp Gln Trp Asn Ile Pro Ala Ser Gln Thr  
 565 570 575

His Leu Ala Ser Asp Ser Arg Ser Lys Gly Glu Pro Arg Asp Ser Gly  
 580 585 590

Thr Leu Gln Ser Gln Glu Ala Lys Ala Val Lys Lys Thr Ser Leu Phe  
 595 600 605

186

Glu Glu Asp Lys Glu Asp Asp Leu Phe Ala Ile Ala Lys Asp Ser Gln  
 610 615 620

Lys Lys Thr Gln Arg Val Ser Leu Leu Phe Glu Asp Asp Val Asp Ser  
 625 630 635 640

Gly Gly Ser Leu Phe Gly Ser Pro Pro Thr Ser Val Pro Pro Ala Thr  
 645 650 655

Lys Lys Lys

<210> 283

<211> 182

<212> PRT

<213> Homo sapiens

<220>

<221> SITE

<222> (22)

<223> Xaa equals any of the naturally occurring L-amino acids

<400> 283

Phe Leu Pro Asp His Pro Ala Lys Pro Pro Ser Ser Leu Val His Ser  
 1 5 10 15

Pro Phe Val Phe Gly Xaa Pro Leu Ser Phe Gln Gln Pro Gln Leu Gln  
 20 25 30

Lys Ser Pro Ser Arg Asn Leu Ala Ser Arg Glu Arg Ile Tyr Lys Asn  
 35 40 45

Tyr Gly Val Ala Gly Pro Ala Ser Ala Leu Ser Ser Leu Ser His Lys  
 50 55 60

Leu Lys Gly Asp Arg Gly Asn Ile Ser Thr Ser Ser Lys Pro Ala Ser  
 65 70 75 80

Thr Ser Gly Lys Ser Glu Leu Ser Ser Lys His Ser Arg Ser Leu Lys  
 85 90 95

Pro Asp Gly Arg Met Ser Arg Thr Thr Ala Asp Gln Lys Lys Pro Arg  
 100 105 110

Gly Thr Glu Ser Leu Ser Ala Ser Glu Ser Leu Ile Leu Lys Ser Asp  
 115 120 125

Ala Ala Lys Leu Arg Ser Asp Ser His Ser Arg Ser Leu Ser Pro Asn  
 130 135 140

187

His Asn Thr Leu Gln Thr Leu Lys Ser Asp Gly Arg Met Pro Ser Ser  
 145 150 155 160

Ser Arg Ala Glu Ser Pro Gly Pro Gly Ser Arg Leu His Leu Leu Ser  
 165 170 175

Gln Arg Leu Ser Gln Gln  
 180

&lt;210&gt; 284

&lt;211&gt; 60

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (22)

&lt;223&gt; Xaa equals any of the naturally occurring L-amino acids

&lt;400&gt; 284

Phe Leu Pro Asp His Pro Ala Lys Pro Pro Ser Ser Leu Val His Ser  
 1 5 10 15

Pro Phe Val Phe Gly Xaa Pro Leu Ser Phe Gln Gln Pro Gln Leu Gln  
 20 25 30

Lys Ser Pro Ser Arg Asn Leu Ala Ser Arg Glu Arg Ile Tyr Lys Asn  
 35 40 45

Tyr Gly Val Ala Gly Pro Ala Ser Ala Leu Ser Ser  
 50 55 60

&lt;210&gt; 285

&lt;211&gt; 60

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 285

Leu Ser His Lys Leu Lys Gly Asp Arg Gly Asn Ile Ser Thr Ser Ser  
 1 5 10 15

Lys Pro Ala Ser Thr Ser Gly Lys Ser Glu Leu Ser Ser Lys His Ser  
 20 25 30

Arg Ser Leu Lys Pro Asp Gly Arg Met Ser Arg Thr Thr Ala Asp Gln  
 35 40 45

Lys Lys Pro Arg Gly Thr Glu Ser Leu Ser Ala Ser  
 50 55 60

&lt;210&gt; 286

&lt;211&gt; 62

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 286

Glu Ser Leu Ile Leu Lys Ser Asp Ala Ala Lys Leu Arg Ser Asp Ser  
1 5 10 15

His Ser Arg Ser Leu Ser Pro Asn His Asn Thr Leu Gln Thr Leu Lys  
20 25 30

Ser Asp Gly Arg Met Pro Ser Ser Ser Arg Ala Glu Ser Pro Gly Pro  
35 40 45

Gly Ser Arg Leu His Leu Leu Ser Gln Arg Leu Ser Gln Gln  
50 55 60

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US00/08979

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C07K 14/47; C12N 5/10, 5/16, 15/12, 15/63, 15/64

US CL : Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/350, 536/23.1, 23.5, 24.3, 24.31; 435/69.1, 71.1, 71.2, 471, 325, 252.3, 254.11, 320.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched  
NONEElectronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
WEST, CAS ONLINE, MEDLINE

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- A	WO 98/04287 A1 (CENTER FOR CLINICAL & BASIC RESEARCH) 05 February 1998 (05.02.98), see entire document, especially pages 23-25.	1-3, 7-11, 14-16 ----- 4-6, 12, 21

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*B* earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*g* document member of the same patent family
*O* document referring to an oral disclosure, use, exhibition or other means	
*P* document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search  
13 JUNE 2000Date of mailing of the international search report  
27 JUL 2000Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

PREMA MERTZ

Telephone No. (703) 308-0196



# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US00/08979

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
  
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application; as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
  
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:  
1-12, 14-16, 21

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.  
☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US00/08979

## A. CLASSIFICATION OF SUBJECT MATTER:

US CL :

530/350, 536/23.1, 23.5, 24.3, 24.31; 435/69.1, 71.1, 71.2, 471, 325, 252.3, 254.11, 320.1

## BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claims 1-12, 14-16, 21, drawn to a nucleic acid of SEQ ID NO:11 encoding a protein of SEQ ID NO:87, a vector, a host cell, a method of making the protein and the protein of SEQ ID NO:87.

Group II, claim 13, drawn to an antibody that binds the protein of SEQ ID NO:87.

Group III, claim 17, drawn to a method of treating a condition comprising administering the protein of SEQ ID NO:87.

Group IV, claim 18, drawn to a method of diagnosing a pathological condition using the polynucleotide encoding a protein of SEQ ID NO:87.

Group V, claim 19, drawn to a method of diagnosing a pathological condition by determining the amount of protein of SEQ ID NO:87.

Group VI, claim 20, drawn to a method of identifying a binding partner of the protein of SEQ ID NO:87.

Group VII, claims 22-23, drawn to a method of identifying an activity in a biological assay.

Group VIII, claim 17, drawn to a method for treating a condition comprising administering the nucleic acid of SEQ ID NO:11.

The inventions listed as Groups I-VIII do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Pursuant to 37 C.F.R. § 1.475 (d), the ISA/US considers that where multiple products and processes are claimed, the main invention shall consist of the first invention of the category first mentioned in the claims and the first recited invention of each of the other categories related thereto. Accordingly, the main invention (Group I) comprises the first-recited product, a nucleic acid encoding a protein of SEQ ID NO:87, a vector, a host cell, a method of making the protein of SEQ ID NO:87, and the protein of SEQ ID NO:87. Further pursuant to 37

C.F.R. § 1.475 (d), the ISA/US considers that any feature which the subsequently recited products and methods share with the main invention does not constitute a special technical feature within the meaning of PCT Rule 13.2 and that each of such products and methods accordingly defines a separate invention.

This application contains claims directed to more than one species of the generic invention. These species are deemed to lack Unity of Invention because they are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for more than one species to be searched, the appropriate additional search fees must be paid. The species are as follows:

the polynucleotides set forth in SEQ ID NO:11-86 encoding the polypeptides set forth in SEQ ID NO:87-162.

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